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HILGARDIA

EXPLANATORY NOTE

With the completion of number twenty of the *Technical Papers* of this institution, the publication of which was commenced in January, 1923, this title has been abandoned and in the future the general type of articles formerly issued in the *Technical Papers* will be published under the title HILGARDIA. The numbers will be paged consecutively and as a volume of reasonable length is completed, a title page, table of contents, and general index will be prepared for each volume.

The chief reason for this change is the cumbersome title of the technical series, it being necessary to quote in full or by abbreviation of the major words the full title, which originally was: "University of California Publications. The Agricultural Experiment Station of the College of Agriculture, Technical Paper No. —." Due to policies adopted by the University Press it became necessary to change this title and the last few numbers were issued under the equally cumbersome title: "University of California, College of Agriculture, Agricultural Experiment Station, Berkeley, California, Technical Paper No. —." The advantages of a title consisting of a single word are manifest.

The title HILGARDIA has been selected to commemorate the services of Doctor Eugene Woldemar Hilgard (1833–1916), who organized the Agricultural Department of the University of California, and who founded the Agricultural Experiment Station in 1875.

President Benjamin Ide Wheeler at the Memorial Services held in honor of Doctor Hilgard, January 30, 1916, stated regarding his services:*

"Eugene Woldemar Hilgard has kept the faith. He has lived among his fellow men in active respect for the principles of order and authority. He has built his life into one of the most institutional forms of human society. He has been a gentleman. He has been true to the best methods and standards of the science in whose fields he has toiled. He has been loyal to the best traditions and standards of academic life."

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* Addresses at memorial services in honor of Dr. E. W. Hilgard, University of California, January 30, 1916. Univ. Calif. Chronicle 18:159–190. 1916. Reprinted by the University of California, Agricultural Experiment Station, under the title: In Memoriam, Eugene Woldemar Hilgard, 1–50. 1916.

Doctor E. W. Allen,* Chief of the Office of Experiment Stations, United States Department of Agriculture, in speaking of Doctor Hilgard's work states:

"The death of Dr. E. W. Hilgard, of California, closes a notable career of service to agriculture, both in length and in accomplishment. It marks the passing of the last of the earlier group of pioneers in agricultural education and research. The work he did dealt with the very fundamentals of agricultural advancement, at a period when men saw the needs less clearly and few were qualified to carry the work forward. Gauged by the time and opportunity, it will remain a great work. Who shall attempt to measure the result of it, or the influence of the high standards he set?"

It seems fitting and appropriate that the name HILGARDIA should be selected as a title of a serial publication of the California Agricultural Experiment Station in which is to be presented the results obtained by members of the staff from painstaking fundamental research on problems related to agriculture. This was the type of agricultural research for which Doctor Hilgard stood. Prominent biologists and patrons of science have had scientific periodicals named in their honor in such title as Addisonia, Bonplandia, Broteria, Candollea, Cassinia, Grevillea, Linnaea, Hedwigia, Malpighia, Muhlenbergia, Teysmannia, Torreya, Treubia, and Webbia. No valid reason exists why such an honor should not be extended to an agriculturist noted for his fundamental work, although the selection of such a title is an innovation in Agricultural Experiment Station literature. It is hoped that the articles that will appear in HILGARDIA, which will be of the same general type as those formerly issued in the series known as *Technical Papers,* will measure up to the high standards Doctor Hilgard set.

E. D. MERRILL,
Dean, College of Agriculture, and
Director of the Agricultural Experiment Station,
Berkeley, California.

April 1, 1925.

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* Editorial in Experiment Station Record 34:301. 1916. In Memorian, Eugene Woldemar Hilgard, 35. 1916.

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FRUIT-BUD DIFFERENTIATION IN DECIDUOUS FRUITS

BY

WARREN P. TUFTS AND E. B. MORROW

Fruit-bud formation, upon which fruit production is dependent, is undoubtedly influenced by such orchard practices as pruning, irrigation, and cultivation. For a successful study of the influence of these various practices upon fruit-bud formation, therefore, an intimate knowledge of the time of differentiation must be available. This paper is the report of studies which have been made under different California conditions over a period of nine years.

TIME OF FRUIT-BUD DIFFERENTIATION

It had been known in a general way that the flowers producing fruit in any year were formed some time during the preceding growing season, but it remained for Goff[6] to recognize definitely the initial stages of flower-bud formation in deciduous orchard fruits. He determined by morphological studies the time when differentiation into flower-buds first occurs and traced the successive stages of development until the unfolding of the blossoms in the spring.

Differences amounting to several days or weeks have been found to occur in the date of the initiation of fruit-bud formation with regard to both climatic influences, and to varieties and types of fruit.

Goff,[6] in a comparison of apple varieties, found a variation of as much as five weeks in the time of flower-bud formation.

Kramer[10] worked with several varieties each of the apple, pear, and cherry and found marked varietal differences, especially in the apple and pear. Little or no variation occurred in the cherry varieties studied. Kramer's work was conducted at Oppenheim, Germany.

Bradford,[1] working in Oregon, found that some varieties differed both in the date of differentiation and in subsequent stages of summer and fall development. Rather wide differences, depending upon the position of buds on the tree, were found to occur in the time of fruit-bud inception. In the Yellow Newtown, buds borne on spurs that had previously fruited, differentiated fully a month ahead of those borne terminally on one-year wood.

Magness[12] found that the initial stages in axillary buds of the apple occurred about one month later than in spur-buds on the same tree. He concluded that the difference between spur-buds and axillary buds seemed to be in degree of development and not in method.

Walker,[15] working under the direction of the senior author of this paper, found that, in 1915, apricot spur-buds differentiated about twenty to twenty-four days earlier than buds on shoots which were largely vegetative in character. During the summer of 1916, however, the difference in the time of differentiation was only from six to eight days.

Wiggans,[16] working also in California, determined the time of differentiation of the Bartlett pear and Royal apricot fruit-buds under the influence of three different sets of conditions, as follows:

1. Regional differences—a comparison of:
    a. Coastal valleys—mild equable climate; average rainfall, thirty inches; elevation, a little above sea level.
    b. Interior valleys—hot dry summers of low humidity; somewhat colder in winter than coastal valleys; average rainfall, about sixteen inches; elevation, a little above sea level.
    c. Foothills—mild climate; summer temperature about the same as coastal valleys; winter temperature somewhat colder than interior valleys; rainfall, about forty inches; elevation, 3000 feet.
2. Heavy vs. light pruning.
3. Irrigation vs. no irrigation.

Wiggans' results are presented in a graphic way in plates 1 and 2; his conclusions from the one season's work were as follows:

"1. Pear fruit-buds begin to differentiate at approximately the same date under coastal valley, interior valley, and foothill conditions.

"2. Apricot fruit-buds begin to differentiate at approximately the same date under coastal valley, interior valley, and foothill conditions.

"3. The high altitude of the foothills seems to have a retarding influence on fruit-bud development until the middle of September, when development becomes quite rapid.

"4. The humid coastal influence apparently stimulates rapid development of pear buds after differentiation. This is not the case with apricots until October, when development becomes extremely rapid and the buds go into the winter at a more advanced stage than is found under either interior valley or foothill conditions.

"5. The dry hot interior valley appears to induce a steady, uniform development of both pear and apricot fruit-buds; however, these have not reached the advanced stage of development by early winter that buds from the coastal valley and foothills have attained.

"6. The inception of fruit-bud differentiation seemingly is not influenced to any extent by either heavy or light dormant pruning. Light pruning perhaps tends to induce a slightly more rapid development for six to eight weeks after fruit-bud differentiation of the pear.

"7. Irrigation shows a tendency to retard fruit-bud differentiation and development.

"8. Environmental conditions during the winter, as found in the principal fruit growing districts of California, apparently do not exert a checking influence upon fruit-bud development of the pear and apricot."

Plates 1 and 2 present the above facts in graphic form.

METHODS USED IN THIS INVESTIGATION

Collection and Preservation of Material.—For the sake of brevity, no attempt is made at this time to describe the collection of materials which was made each season from 1915 to 1923, inclusive, except to say that with minor variations, these collections were identical with those which are here recorded for the 1923 season.

During 1923, all the material studied was collected on the University Farm at Davis, California. Probably various differences in soil, climate, and cultural treatments bring about minor differences in the time of differentiation; however, the work done by Wiggans tends to show that, at least for California and for the varieties studied, the results obtained from materials collected at the University Farm can be taken as generally applicable to the leading deciduous fruit sections of the state.

Material for study was secured at intervals of approximately ten days from May 18, up to the middle of August, 1923. From then until early November, collections were made every two weeks and subsequently at somewhat wider intervals until December 22. Collections were made from the following fruits and varieties:

Fruit	*Species*	*Variety*
Almond	*Prunus amygdalus*	Nonpareil
Apple	*Pyrus malus*	Gravenstein
Cherry (sour)	*Prunus cerasus*	Early Richmond
Cherry (sweet)	*Prunus avium*	Napoleon (Royal Ann)
Peach	*Prunus persica*	Elberta
Pear	*Pyrus communis*	Bartlett
Plum (European)	*Prunus domestica*	French (prune)
Plum (Japanese)	*Prunus salicina* × *Prunus simonii*	Wickson

Only spur-buds were collected from the almond, apple, apricot, cherry, pear, and plum, while from the peach, buds were collected from the current season's shoots only. At each collection approximately forty buds, well distributed throughout the tree, were taken and immediately put into the formalin-alcohol killing and fixing solution* in which they were preserved until sectioning could be accomplished.

Sectioning.—With the apricot, cherry, plum, and peach, the paraffin method of embedding as outlined by Chamberlain[2] was found reasonably satisfactory as a means of preparing the buds for sectioning. With the apple and pear, however, the paraffin method proved unsatisfactory because of the extremely hairy nature of the material. Even with the careful trimming off of the bud scales or other woody portions and the removal of a large number of hairs under the dissecting microscope, infiltration was difficult and the sections broke on the microtome blade.

Much time was spent in an effort to find a satisfactory method of sectioning this refractory material. Chloroform was tried as a clearing agent, but buds cleared in chloroform sectioned little better than those cleared in xylol. Buds were also soaked in hydrofluoric acid for a period of ten days to two weeks before being embedded in paraffin, but this too gave only indifferent results.

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* Killing and fixing solution: Alcohol, 50 per cent..........94 cc. ✓
Formalin, 40 per cent.......... 6 cc. ✓

A considerable amount of effort was spent in trying to adapt a combination of the paraffin and celloidin methods to the material in hand. The first method tried was that reported by Kornhauser,[9] but the infiltration process is long, and the results secured in sectioning were not satisfactory. An attempt was made to shorten the celloidin infiltration by using Gilson's[5] "Rapid Process" method, but few successful sections were thus obtained. An abridgment of the Kornhauser method by de Zeeuw[3] of Michigan gave reasonably satisfactory results. By this method the material was infiltrated in medium celloidin and then dropped directly into chloroform. In preparation for the paraffin infiltration and embedding, Apathay's oil mixture was omitted and chloroform was substituted for benzol.

De Zeeuw's method is short and convenient to use, and offers many of the advantages of the celloidin and paraffin methods with few of the disadvantages of either. He reports that with ordinary fixatives the sections sometimes wash off the slides when the celloidin is removed; he recommends, therefore, Szomobathy's gelatin fixative wherever this difficulty is experienced. However, in the present investigation, no trouble of this kind arose while using an albumen-glycerine fixative, when the paraffin was dissolved in xylol and the celloidin in ethyl alcohol.

Staining.—A combination stain of safranin and Delafield's haemotoxylin gave the most satisfactory results. The sections were over-stained in safranin and de-stained in acid alcohol; then over-stained in haemotoxylin and reduced by placing in water to which had been added a few drops of concentrated hydrochloric acid.

PRESENTATION OF RESULTS

Goff[6] considered that slight irregularities in the growing point or crown of the bud were the first evidences that differentiation had taken place. He found that in the individual flower-bud, the calyx was first to be formed, and concluded that "in the normal order of development the corolla originates next after the calyx, and is followed in turn by the stamens and pistil."

Drinkard[4] also considered that corrugations on the crown of the bud were the first morphological evidences that a differentiation into flower-buds had taken place.

Kraus,[11] in a study of the gross morphology of the apple, has the following to say with regard to the first indications of a change into a flower-bud: "From a study of many sections and dissections, it is found that the first observable indication of the flower is the more or less thickening of the axis. Minute bracts, in the axils of which are formed blunt protuberances, arise from it in a very close spiral. The tip of the axis never loses its identity, but on the contrary enlarges considerably and always develops slightly in advance of the protuberances immediately below it. Later, these protuberances develop into definite individual flowers."

Bradford,[1] in writing of the apple, says that "the first evidence of fruit-bud formation lies in the rapid elevation of the crown into a narrow conical form, rounded at the apex, with the fibro-vascular connections and pith areas advancing concurrently. In the axils of the young leaves, already noted in connection with the differentiated bud, appear other protuberances which soon become blunt at the tip, while at the same time other leaf protuberances appear in their axils. The apical protuberance is differentiated last, but when it does take shape it is already larger than those previously laid down, apparently appropriating a larger mass of tissue in its formation."

In this investigation the authors have considered the definite broadening and thickening of the floral axis as evidence of the first differentiation of the floral parts. The formation of slight proturberances which eventually become calyx, corolla, stamens, and pistil follows almost immediately, varying somewhat in detail, of course, with the different species. The detailed comments on the specific fruits apply particularly to the 1923 season unless otherwise mentioned.

Almond.—The Nonpareil almond showed no signs of differentiation until September 1 (pl. 3, fig. 3). Several buds from this collection showed elongated crowns, flattened on top, indicating that differentiation had already occurred. In the case of buds from the collection made on September 15 the crown had thickened considerably, and the sepal primordia had begun to push up from the sides. Salinger,[13] working in California during the 1915 season, found that differentiation had occurred in the I.X.L. variety of almond on August 18.

Apple.—In the Gravenstein apple some of the buds from the collection made on June 11 had already begun to develop into flower-buds (pl. 4, fig. 1). By June 20, the apical flower had enlarged considerably and was showing prominent sepal primordia, and the adjacent flowers were clearly visible. Growth was rapid during the next few days and the petal primordia had appeared on July 11. Buds collected on August 17 showed stamen primordia, and by October 13 the early stages of pistil formation were plainly visible. Later growth was apparently somewhat slower, few changes occurring from November 10 to December 22.

Apricot.—The Royal apricot showed first signs of differentiation on August 10 (pl. 5, fig. 1). At this time the axis of the crown was considerably thickened, and the sepal primordia were beginning to arise from the sides. Walker[15] observed the initial stage on August 4, 1915, and August 10, 1916, and Wiggans[16] on August 10, 1922. The gradual development of the flower-bud is shown (pl. 5, figs. 2–8).

Cherry.—The first collection of Early Richmond cherry (*Prunus cerasus*) was made on July 12. At this time the earlier stages of the individual flower-buds had appeared in the form of prominent protuberances (pl. 6, fig. 1). By August 10, both sepal and petal primordia were plainly visible, and buds from the collection of September 1 showed the earlier stages of stamen and pistil formation. All flower parts had very nearly assumed their final form by September 29, and the ovarian cavity had appeared on October 13. Growth was relatively slow from early November until December 22.

In the Napoleon (Royal Ann) cherry (*Prunus avium*), the first clear evidences of differentiation appeared on July 3 (pl. 7, fig. 1). By July 30 the sepal protuberances were beginning to push up from the sides of the buds, and on August 17 both petal and stamen primordia had appeared, and the pistil was beginning to grow from the base of the flower-cup. All flower parts had assumed their final form by late September, little development occurring from this time until late in the dormant season.

Peach.—In the Elberta peach, differentiation had taken place by July 30 (pl. 8, fig. 2). On August 10 the sepal primordia were beginning to appear and by August 17 the earlier stages of petal formation were clearly visible.

Pear (pl. 9).—In collections from the Bartlett pear made on June 21, some of the buds showed the earlier stages of fruit-bud formation. By July 3 the axial flower-buds had appeared. Growth was gradual from this time until the latter part of November; few gross changes took place from November 30 until early spring. The results here reported complete three seasons observations in California of fruit-buds of the Bartlett pear. In 1915 Henderson[8] found first evidences of differentiation on July 3, and Wiggans[16] working during the 1922 season, found that differentiation had occurred on July 4. Although some of the buds from the collection of June 21, 1923, showed evidences of flower-bud formation, it is quite probable that the percentage of buds differentiated at this time is very small, and fruit-bud differentiation in the Bartlett pear under California conditions may be said to begin during early July.

Plum (pl. 10).—Buds from the French prune (*Prunus domestica*) collected on August 10 showed no signs of differentiation, but those collected on August 17 showed individual flower-buds. Generally speaking, the stages of growth were somewhat variable on the same date. This may be partly accounted for by the fact that the tree from which the buds were collected was practically defoliated in August by a severe infestation of red spider. It is of interest to note that Hartwell[7] found the first observable stages of differentiation in the French prune to occur six weeks earlier during 1920 than was the case in 1923.

Bud specimens taken on August 10 (pl. 11, fig. 1) from the Wickson plum (*Prunus salicina* × *Prunus simonii*), showed the bud scales still arising from the sides of the crown; no evidences of differentiation were found. By September 1 the individual flower-buds had appeared and the sepal primordia were pushing up from the sides of the bud. In the collection of October 13, the earlier stages of petal, stamen, and pistil formation were visible, and by December 22 all flower parts had assumed final form. Trunk[14] found that the Wickson plum showed first evidences of differentiation on July 31 during the 1915 season.

Table 1 gives in condensed form the findings of various investigators, including those reported here, as to initiation of flower-bud formation in deciduous fruits.

TABLE 1

Fruit	Variety	Differentiation first noted	Locality	Investigator
Almond	I. X. L.	August 18, 1915	California	Salinger
	Nonpareil	September 9, 1923	California	Tufts and Morrow
Apple	Hoadley	June 30, 1899	Wisconsin	Goff
	Oldenburg	June 30, 1909	Virginia	Drinkard
	Yellow Newtown	Early July, 1912	Oregon	Bradford
	Gravenstein	June 11, 1923	California	Tufts and Morrow
Apricot	Royal	August 4, 1915	California	Walker
	Royal	August 10, 1916	California	Walker
	Royal	August 11, 1922	California	Wiggans
	Royal	August 10, 1923	California	Tufts and Morrow
Blackberry	Snyder	Late August, 1915	New York	MacDaniels
Cherry	King's Amarelle	July 11, 1899	Wisconsin	Goff
	King's Amarelle	July 5, 1900	Wisconsin	Goff
	Louis Phillippe	July 1, 1909	Virginia	Drinkard
	(No variety named)	Before end of July, 1922	Germany	Kramer
	Early Richmond	July 12, 1923	California	Tufts and Morrow
	Napoleon (Royal Ann)	July 3, 1923	California	Tufts and Morrow
Cranberry	(No variety named)	September 16, 1900	Wisconsin	Goff
Currant	Pomona	July 8, 1900	Wisconsin	Goff
	Black Victoria	August 3, 1900	Wisconsin	Goff
	Cherry Red	Early August, 1915	New York	MacDaniels
Filbert	(No variety named)	Catkins—June 10, 1894 Pistillate flowers—Early September	Germany	Albert
Gooseberry	Downing	August 30, 1900	Wisconsin	Goff
	Houghton	Early August, 1915	New York	MacDaniels
Grape	(No variety named)	Mid-June, 1898	Germany	Behrens
Peach	Bokhara	September 21, 1900	Wisconsin	Goff
	Luster	July 7, 1909	Virginia	Drinkard
	Deming's September	June 14, 1900	Georgia	Quaintance
	Elberta	June 30, 1923	California	Tufts and Morrow
Pear	Wilder Early	July 21, 1899	Wisconsin	Goff
	Wilder Early	September 6, 1900	Wisconsin	Goff
	Kieffer	July 15, 1909	Virginia	Drinkard
	Bartlett	July 3, 1915	California	Henderson
	Bartlett	July 4, 1922	California	Wiggans
	Bartlett	June 21, 1923	California	Tufts and Morrow
Plum	Rollingstone	July 8, 1899	Wisconsin	Goff
	Rollingstone	July 5, 1900	Wisconsin	Goff
	Whitaker (Wild Goose)	September 1, 1909	Virginia	Drinkard
	Japanese	July 14, 1909	Virginia	Drinkard
	Wickson	July 31, 1915	California	Trunk
	Wickson	Mid-August, 1923	California	Tufts and Morrow
Prune	French	June 29, 1920	California	Hartwell
	French	Mid-August, 1923	California	Tufts and Morrow
Raspberry	Cumberland (Black)	October 6, 1915	New York	MacDaniels
	Herbert (Red)	January 11, 1916	New York	MacDaniels
Strawberry	Clyde	September 20, 1900	Wisconsin	Goff

SUMMARY

A study has been made, using approved laboratory methods, of the date of fruit-bud differentiation in some of the principal fruits of temperate climates produced in California. The approximate dates of differentiation are briefly summarized in the following table:

TABLE 2

Fruit	Variety	Date of Differentiation
Almond	Nonpareil	Late August—Early September
Apple	Gravenstein	Mid-June
Apricot	Royal	Early August
Cherry (Sour)	Early Richmond	Early July
Cherry (Sweet)	Napoleon (Royal Ann)	Late June—Early July
Peach	Elberta	Late July
Pear	Bartlett	Late June—Early July
Plum (European)	French	Late July—Early August
Plum (Japanese)	Wickson	Late July—Early August

The following conclusion seems justified:

The date of differentiation may vary somewhat in widely separated regions within any one species, although it seems that under most conditions in California little variation occurs.

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EXPLANATION OF THE PLATES

PLATE 1

Outline drawings of longitudinal sections through Bartlett pear fruit-buds showing the average stages of development at different dates under various climatic environments, soil moistures, and pruning treatments. (From thesis by Wiggans.)

PLATE 2

Outline drawings of longitudinal sections through Royal apricot fruit-buds showing the average stages of development at different dates, under various climatic environments, soil moistures, and pruning treatments. (From thesis by Wiggans.)

PLATE 3

Photomicrographs of longitudinal sections through fruit-buds of the Nonpareil almond (× 40).

Fig. 1. July 30, 1923.
Fig. 2. August 17, 1923.
Fig. 3. September 1, 1923.
Fig. 4. September 15, 1923.

PLATE 4

Photomicrographs of longitudinal sections through fruit-buds of the Gravenstein apple (× 40).

Fig. 1. June 11, 1923.
Fig. 2. July 11, 1923.
Fig. 3. October 13, 1923.
Fig. 4. December 22, 1923.

PLATE 5

Photomicrographs of longitudinal sections through fruit-buds of the Royal apricot (× 40).

Fig. 1. August 10, 1923.
Fig. 2. September 1, 1923.
Fig. 3. October 5, 1915.
Fig. 4. October 30, 1922.

PLATE 5—(*Continued*)

Photomicrographs of longitudinal sections through fruit-buds of the Royal apricot (× 40).

Fig. 5. November 22, 1915.
Fig. 6. January 13, 1916.
Fig. 7. February 10, 1916.
Fig. 8. February 17, 1916.

PLATE 6

Photomicrographs of longitudinal sections through fruit-buds of the Early Richmond cherry (× 40).

Fig. 1. July 12, 1923.
Fig. 2. August 17, 1923.
Fig. 3. September 15, 1923.
Fig. 4. November 30, 1923.

PLATE 7

Photomicrographs of longitudinal sections through fruit-buds of the Napoleon cherry (× 40).

Fig. 1. July 3, 1923.
Fig. 2. July 30, 1923.
Fig. 3. August 17, 1923.
Fig. 4. November 20, 1923.

PLATE 8

Photomicrographs of longitudinal sections through fruit-buds of the Elberta peach (× 40).

Fig. 1. July 20, 1923.
Fig. 2. July 30, 1923.
Fig. 3. August 10, 1923.
Fig. 4. August 17, 1923.

PLATE 9

Photomicrographs of longitudinal sections through fruit-buds of the Bartlett pear (× 40).

Fig. 1. July 21, 1923.
Fig. 2. July 31, 1922.
Fig. 3. November 30, 1923.
Fig. 4. February 28, 1923.

PLATE 10

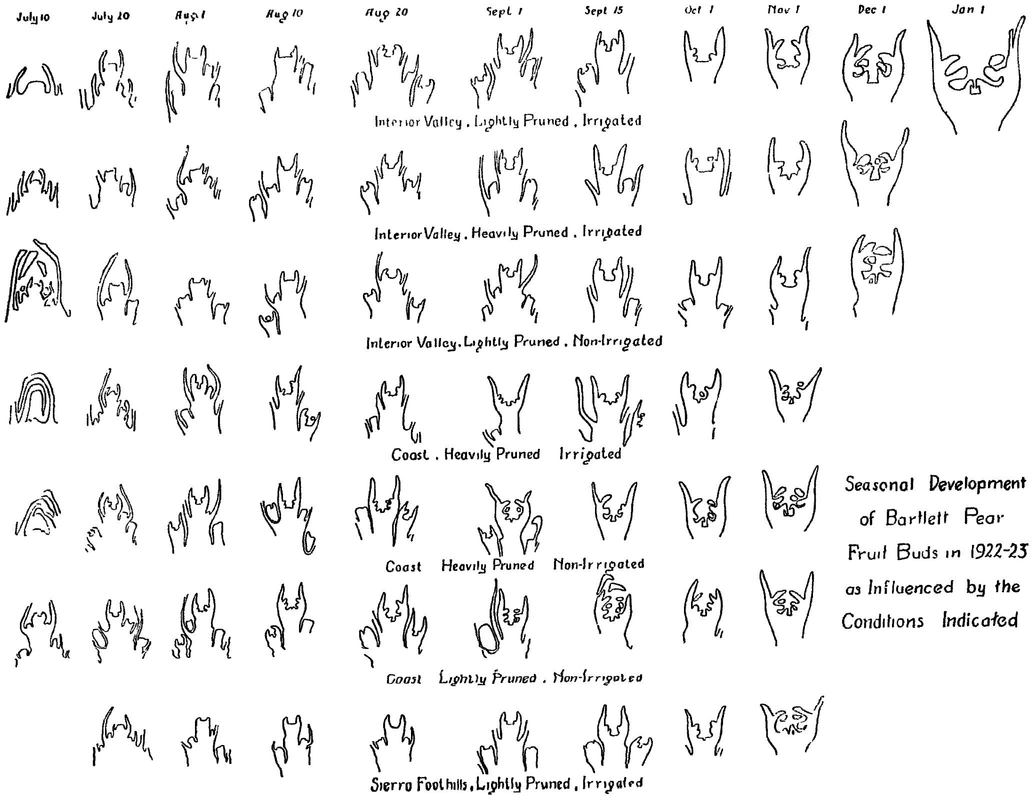
Photomicrographs of longitudinal sections through fruit-buds of the French prune (× 40).

Fig. 1. August 17, 1920.
Fig. 2. September 15, 1920.
Fig. 3. December 7, 1920.
Fig. 4. February 2, 1921.

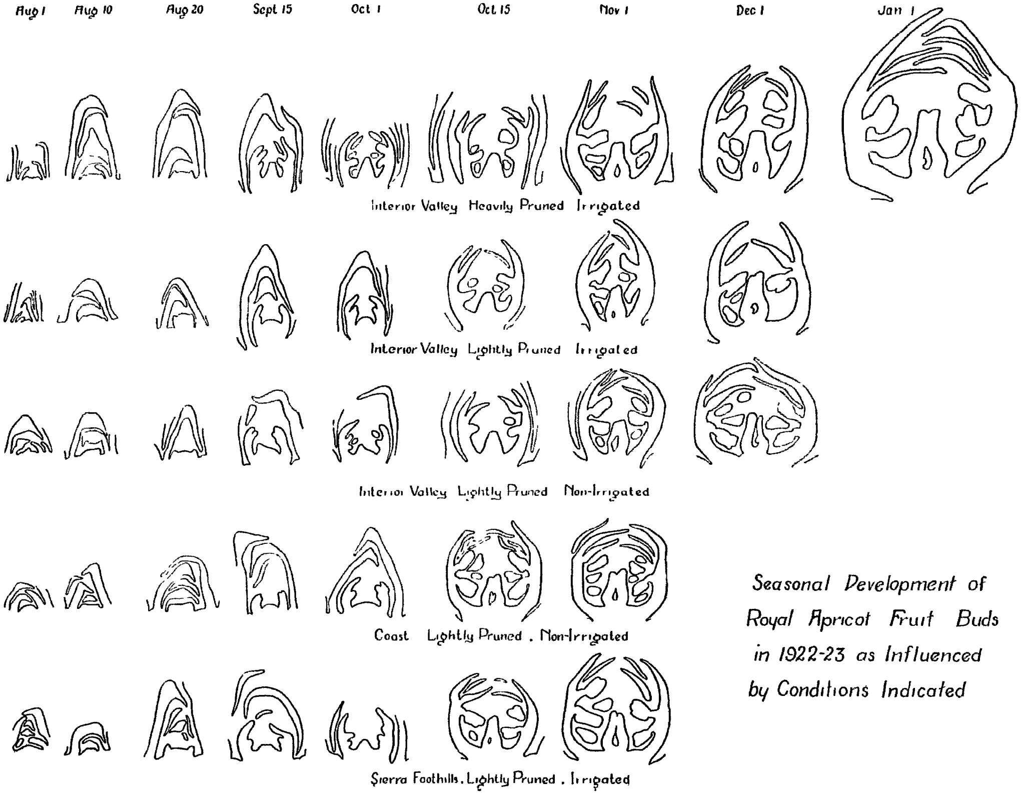
PLATE 11

Photomicrographs of longitudinal sections through fruit-buds of the Wickson plum (× 40).

Fig. 1. August 10, 1923.
Fig. 2. September 1, 1923.
Fig. 3. October 13, 1923.
Fig. 4. December 22, 1923.


July 10
July 20
Aug 1
Aug 10
Aug 20
Sept 1
Sept 15
Oct 1
Nov 1
Dec 1
Jan 1
Interior Valley, Lightly Pruned, Irrigated
Interior Valley, Heavily Pruned, Irrigated
Interior Valley, Lightly Pruned, Non-Irrigated
Coast, Heavily Pruned Irrigated
Coast Heavily Pruned Non-Irrigated
Coast Lightly Pruned, Non-Irrigated
Sierra Foothills, Lightly Pruned, Irrigated

Seasonal Development of Bartlett Pear Fruit Buds in 1922-23 as Influenced by the Conditions Indicated


Aug 1
Aug 10
Aug 20
Sept 15
Oct 1
Oct 15
Nov 1
Dec 1
Jan 1
Interior Valley Heavily Pruned Irrigated
Interior Valley Lightly Pruned Irrigated
Interior Valley Lightly Pruned Non-Irrigated
Coast Lightly Pruned . Non-Irrigated
Sierra Foothills . Lightly Pruned . Irrigated

Seasonal Development of Royal Apricot Fruit Buds in 1922-23 as Influenced by Conditions Indicated

NONPAREIL ALMOND

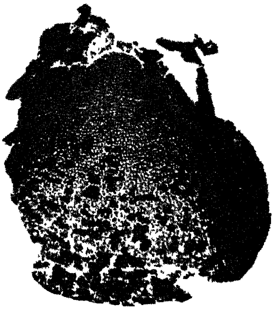


Fig. 1

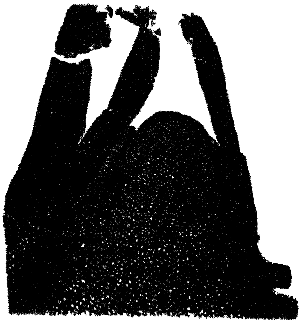
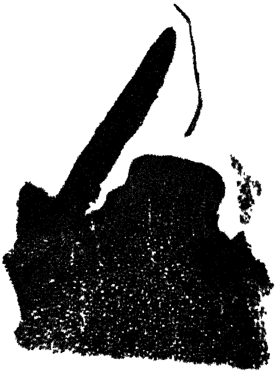


Fig. 2



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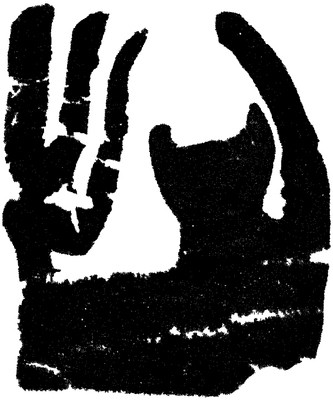
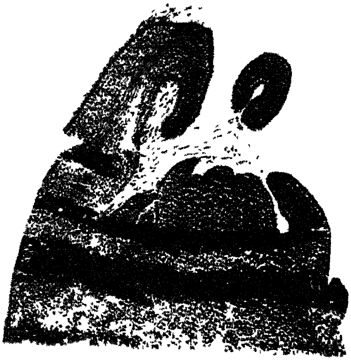


Fig. 4

GRAVENSTEIN APPLE



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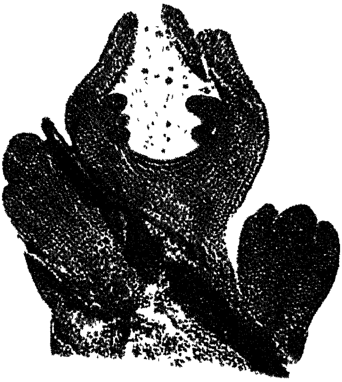

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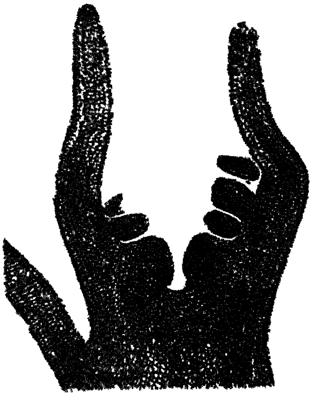

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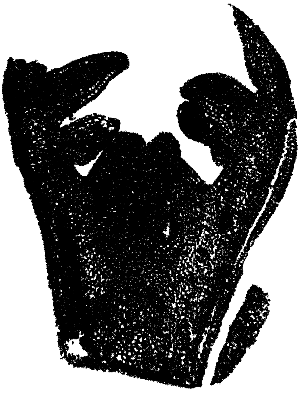
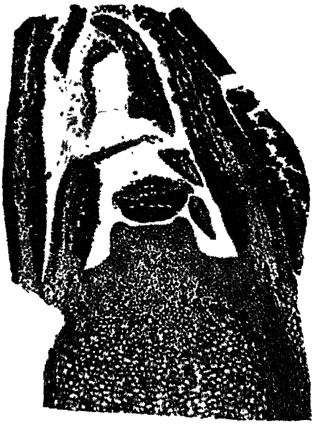


Fig. 4

ROYAL APRICOT



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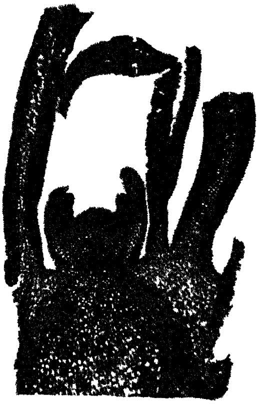

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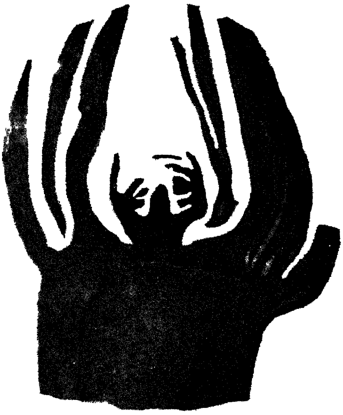

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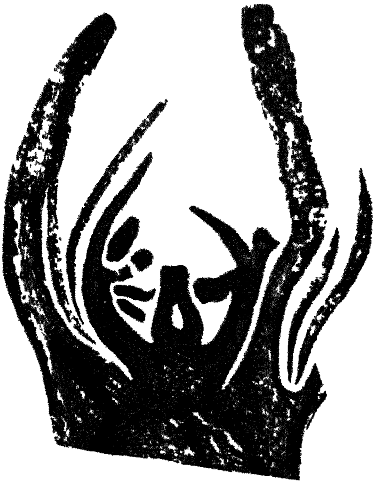

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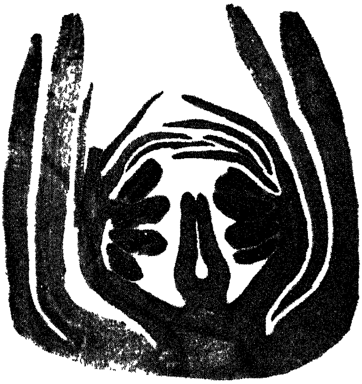
ROYAL APRICOT



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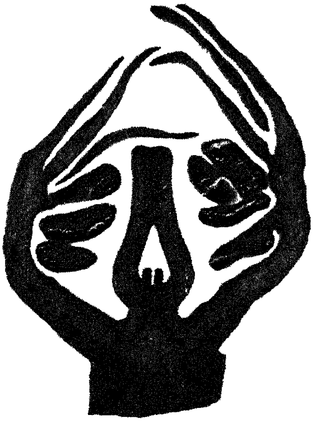

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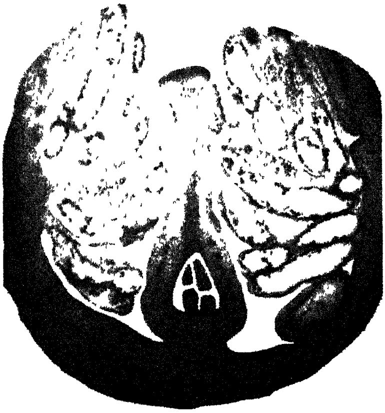

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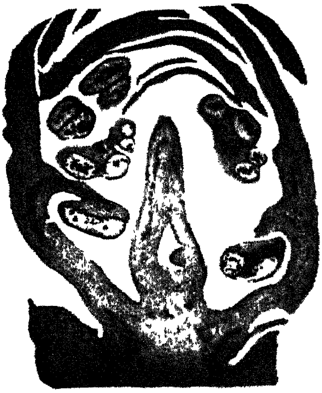
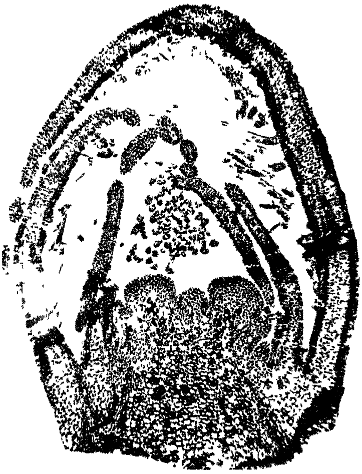


Fig. 8

EARLY RICHMOND CHERRY



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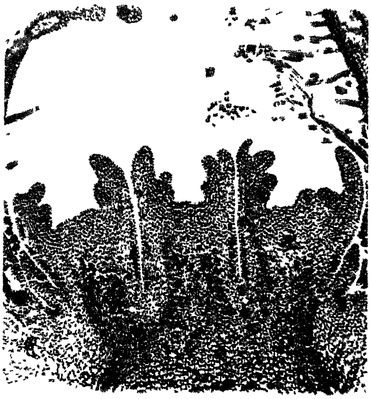

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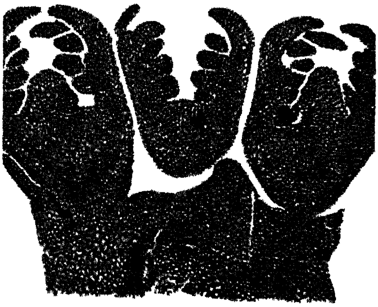

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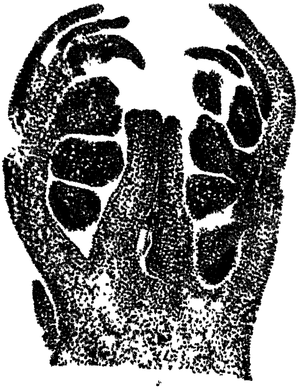
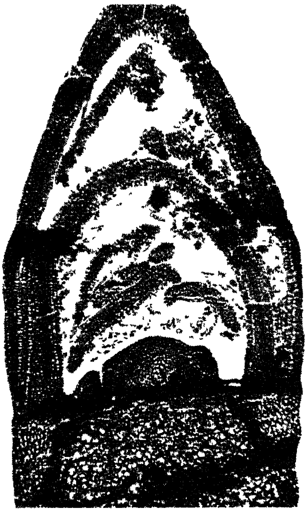


Fig. 4

NAPOLEON CHERRY



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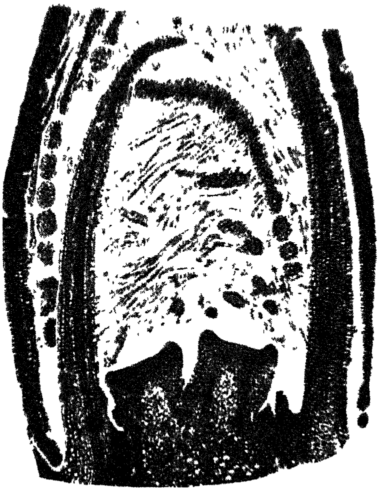

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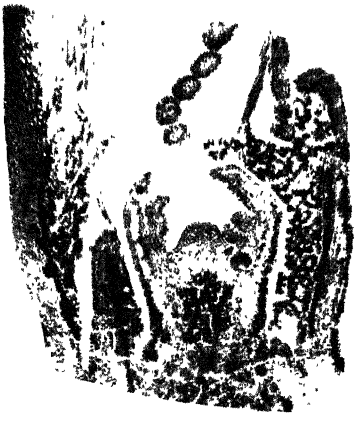

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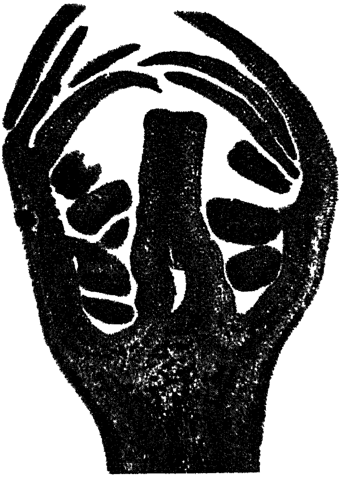
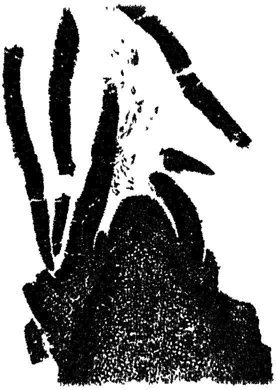


Fig. 4

ELBERTA PEACH



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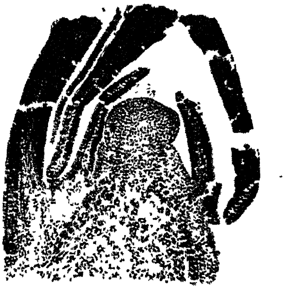

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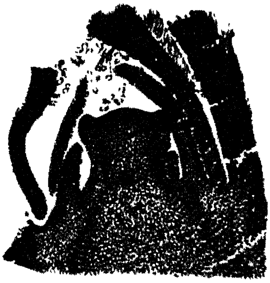

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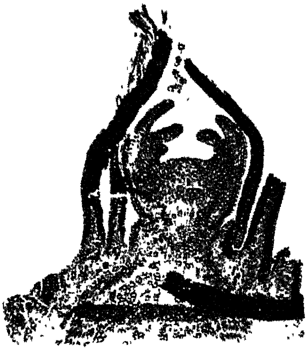


Fig. 4

BARTLETT PEAR

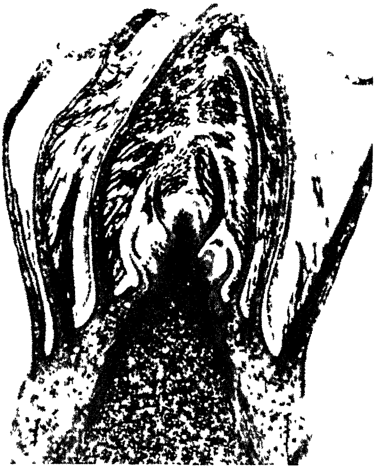
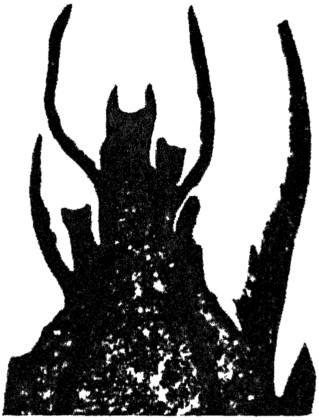


Fig. 1



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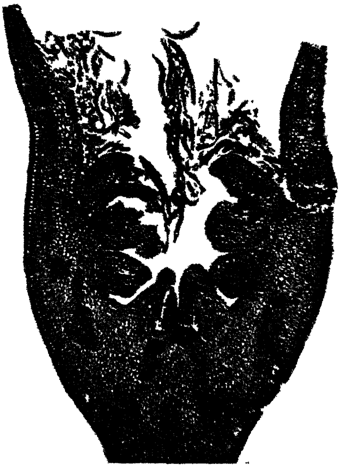

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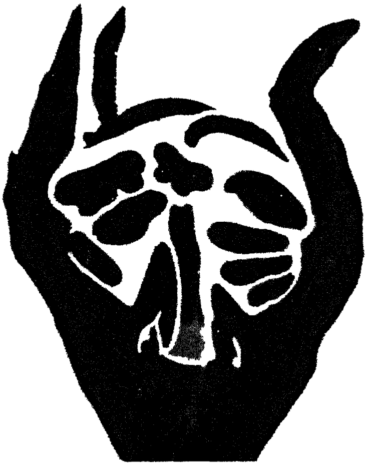
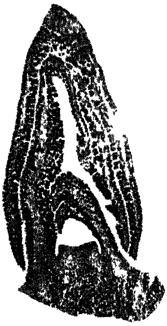


Fig. 4

FRENCH PRUNE



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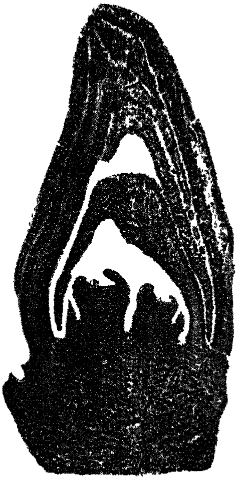

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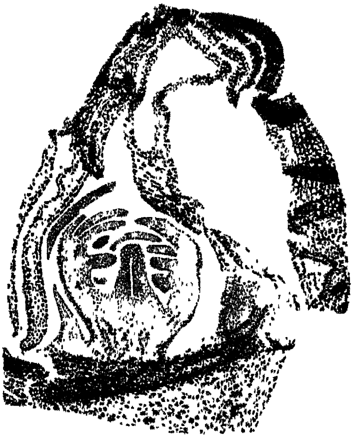

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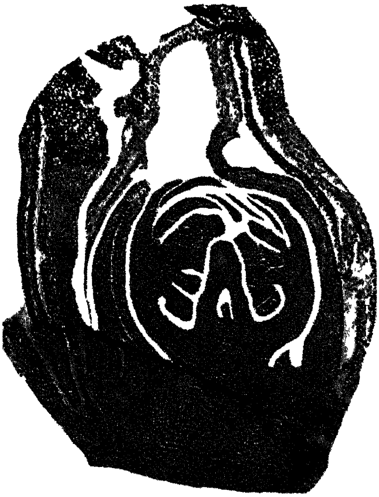
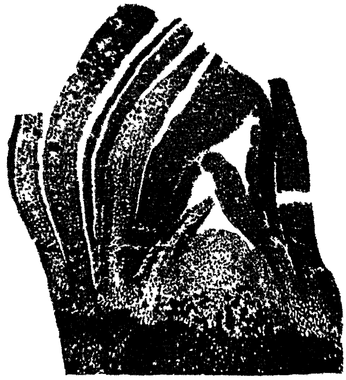


Fig. 4

WICKSON PLUM



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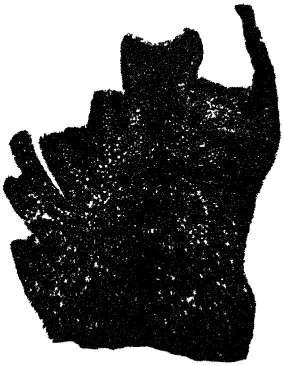

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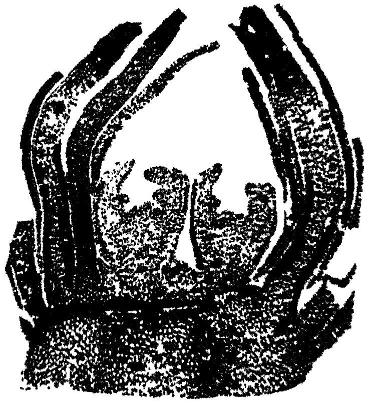

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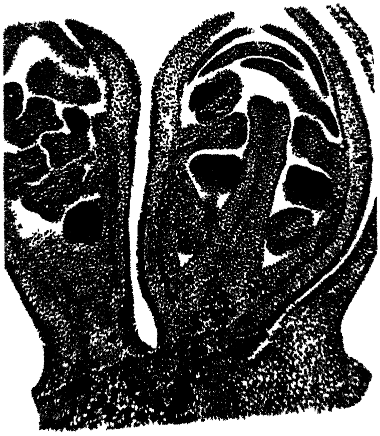

Fig. 4

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THE ANTISCORBUTIC VALUE OF COMMERCIALLY CONCENTRATED ORANGE JUICE

BY

HAROLD GOSS

INTRODUCTION

Concentrated orange juice prepared commercially has only recently been placed on the market. So far as we are aware, all reports on the antiscorbutic property of concentrated juices are based on work with laboratory preparations, except that of Chaney,[1] who reported on the use of a concentrated bottled orange juice as a supplemental lunch for school children and suggested that the favorable results obtained may have been due, in part, to the antiscorbutic principle of the orange juice. It was, therefore, deemed desirable to ascertain whether or not these commercial products retained the antiscorbutic property to as great an extent as did the laboratory preparations.

It was realized as long ago as the sixteenth century that oranges and lemons possessed great value as preventives of scurvy,[2] but at that time little significance was given to this fact and more attention was paid to limes and other fresh fruits. Orange and lemon juice have now been studied more than any other antiscorbutic substance. Limes and lime juice are no longer regarded as excellent sources of vitamin C. Chick, Hume, and Skelton[3] found lime juice to be only one-fourth as potent as lemon juice, while lemon and orange juice are considered equal in value in this respect. Oranges and lemons have the highest known antiscorbutic value and are, therefore, almost always taken as a standard in comparative work.

It has been known for some time that orange juice may be heated to boiling[4] or dried in vacuum under certain conditions and reduced to a powdered form[5] and still retain significant antiscorbutic power. It has been pointed out by other investigators that oxidation is responsible for the rapid destruction of vitamin C in orange juice; such destruction taking place even in the cold when the juice is sufficiently exposed to air or other oxidizing influences. Neutralization of the citric acid has a similar marked effect.[6, 7] Givens and MacCluggage,[5] in their early work in 1919, pointed out the advantages that would follow if some method of concentration were perfected which would not lower the antiscorbutic value of orange and lemon juice. This would make it possible to prepare and preserve these valuable food products from surplus fruit and put them on the market at a comparatively low price. It has been shown by the results of investigations already carried out that concentration of the juice in vacuum under reduced temperatures is a practical commercial method of accomplishing this object.

METHOD OF PROCEDURE

Using fresh orange juice as a standard antiscorbutic and a basal diet of oats, barley, wheat hay, and water, comparative results were obtained by studying the clinical effects on guinea-pigs of the basal diet plus a measured amount of the product studied. An attempt was thus made to secure approximate quantitative results in terms of fresh orange juice, but as others have often noted[7] the individual variations of the animals and the uncertainty of the border line between protection and failure to protect render exact measurement of the results by this method impossible.

The concentrated juices were, after dilution with suitable known quantities of water, administered orally daily in measured amounts, by means of a 5 cc. hypodermic syringe (fig. 1). Very little difficulty was experienced by this method in administering an exact quantity of the juice. It was found that the animals relished all juices after a few trials with the exception of concentrated lemon. By carefully releasing the liquid from the syringe 5 cc. could be administered without loss.

The desiccated orange juice was dissolved in water and then used with the syringe as described above.

The dried whole orange was given in gelatin capsules each containing 0.2 grams of the product, ground as fine as possible in a food chopper.

In all experiments normal, healthy guinea-pigs were used, ranging in weight from 400 to 800 grams. They were kept, two or three together, in metal cages in a sunlit room, protected from draughts and changes in temperature. The bedding used was covered by a layer three or four inches thick of clear wheat hay, which was replenished every three days when the cages were cleaned. Rolled oats, rolled barley, and water were kept before the animals at all times.

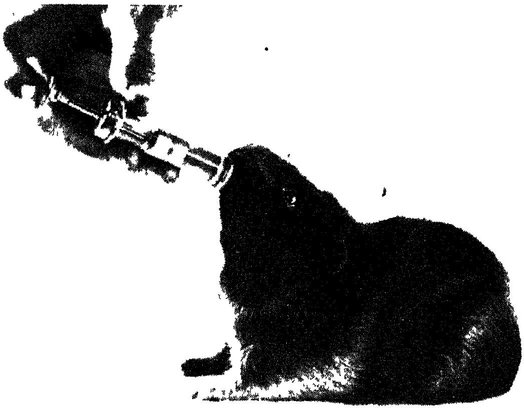

Fig. 1. Method of feeding measured quantity of juice by means of hypodermic pipette

EXPERIMENTAL

Besides the concentrated whole orange juice our study included several other orange and lemon products, prepared recently on a commercial scale. Experimental data is submitted on the following products:

1. Concentrated whole orange juice.
2. Concentrated and clarified orange juice.
3. Desiccated orange juice prepared by a spray process with added cane sugar.
4. Dried whole orange.
5. Concentrated, slightly sweetened lemon juice.

The orange and lemon concentrates were prepared by a standard well-known method of concentration employing glass-lined vacuum pans operated under very high vacuum and low temperature. These products were furnished by the Exchange Orange Products Company at San Dimas, California, and the Exchange Lemon Products Company at Corona, California, two commercial production plants coöperatively owned and operated by members of the California Fruit Growers' Exchange.

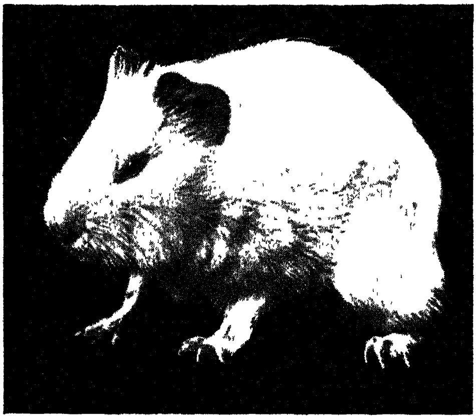

Fig. 2. General condition of scorbutic animal after 30 days on a diet of oats, barley, wheat hay, and water.

The desiccated orange juice was furnished by the Research Laboratory of the Exchange. The dehydrated ground whole orange was submitted by the Laboratory of Fruit and Vegetable Chemistry, U. S. Department of Agriculture, Los Angeles, California.

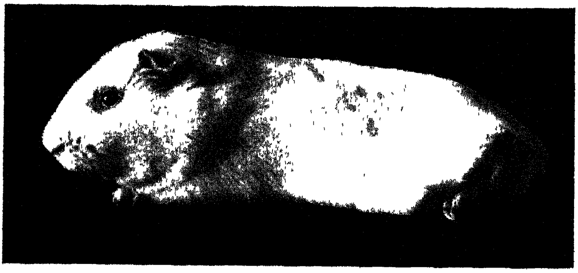

Fig. 3. Guinea-pig suffering from scurvy, showing painful limb extended to relieve pressure.

1. *Basal Diet Alone.*—On the basal diet alone animals usually showed, in about 20 to 25 days, indications of failure of health, usually characterized by weakness, a staring coat (fig. 2), swollen

wrist joints—especially in the young animals—tenderness of the limbs, and signs of pain when handled. Frequently the animal would relieve pain in the affected limb by removing pressure as shown in figure 3. Occasionally the well known "face-ache position" described by Chick, Hume, and Skelton[3] was observed.

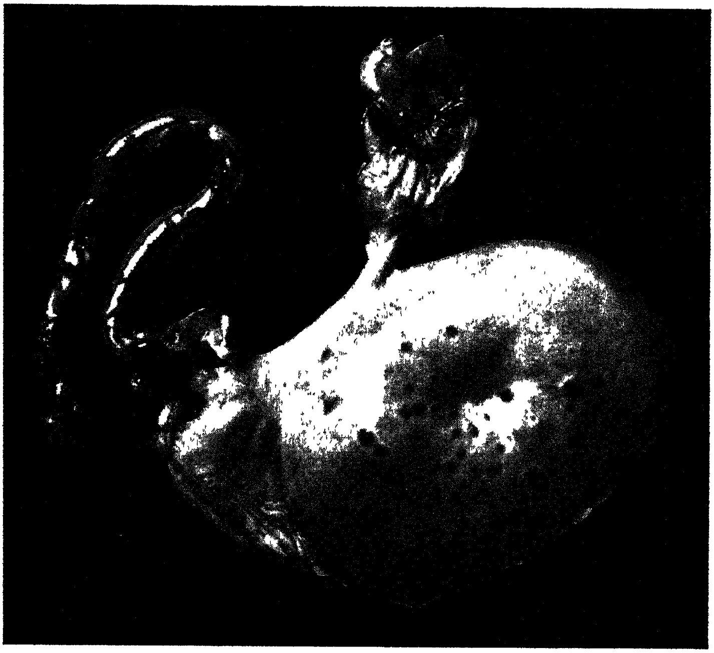

Fig. 4. Stomach of a scorbutic guinea-pig distended as a result of gas, showing hemorrhages on the interior, even before being opened (see fig. 5).

Unless fresh orange juice or grass was given when the symptoms were first noticed the animal would invariably die in 10 days more. Post mortem examinations of these animals gave various pictures described by other investigators, but no complete set of symptoms and lesions were observed in all cases.

The most predominating lesion found in our cases were hemorrhages in various tissues of the body, usually in the stomach and intestines (figs. 4, 5, 6, and 7) and occasionally in the muscles of the hind legs. Frequently enlargements of the costochondral junctions of the ribs were well pronounced (see fig. 8 and compare with fig. 9). The bones were always more or less brittle. In two cases, jaw bones were found fractured. The teeth were usually dull and brittle, the incisors easily yielding to fracture on the ends by the pressure of the finger nail.

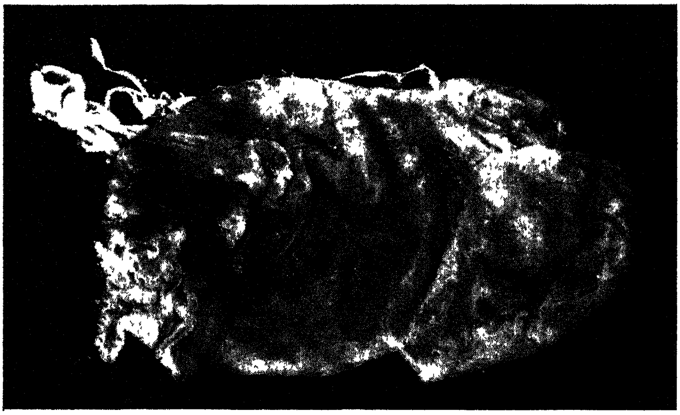

Fig. 5. Appearance of inside lining of same stomach shown in figure 4. Note the numerous hemorrhages dotted through the lining.

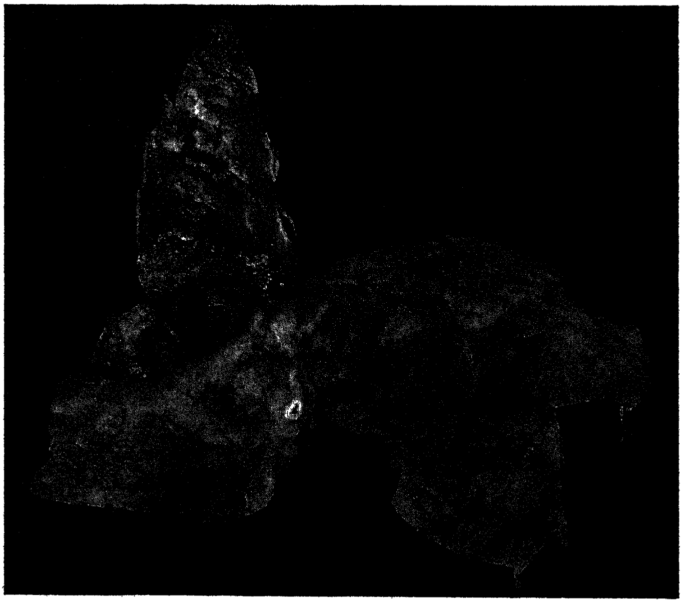

Fig. 6. Severe hemorrhages in the stomach of a guinea-pig which died from scurvy.

The growth curves are not especially significant except to give some idea of the well being of the animals during the test. In nearly all cases symptoms of scurvy were not apparent until after a notable decline in weight was observed.

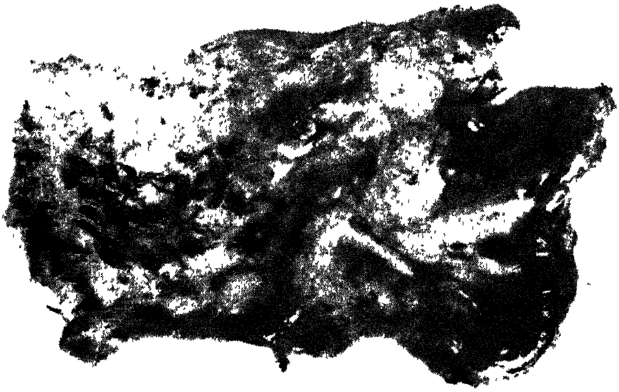

Fig. 7. Hemorrhagic erosions in the stomach lining of a scorbutic guinea-pig. The white ring in the upper center is the pylorus.

When any of the above symptoms developed it was assumed that either no protection was being offered by the substance in question or that the protection was not sufficient.

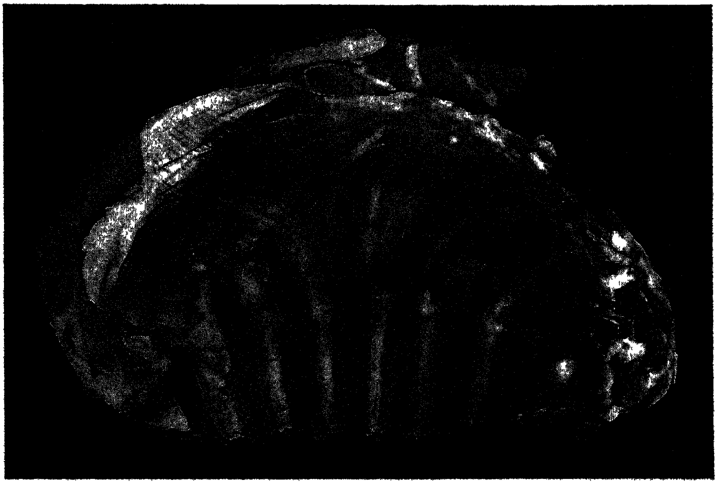

Fig. 8. Section of ribs showing enlarged costochrondral junctions observed in some of the scorbutic animals. Compare with figure 9.

Curative methods are sometimes used in determining the presence of vitamin C but the conditions of the animals were so uncertain that quantitative results were found with difficulty and in many cases not at all. Therefore, more reliance has been placed on the preventive than on the curative method.

2. *Minimum Dose of Fresh Orange Juice.*—When the basal diet was supplemented by 1.5 cc. of fresh orange juice daily, animals were successfully maintained for a period of 93 days, gaining 55 per cent in weight and showing no signs of scurvy at anytime (fig. 10). Other animals fed similarly, except that alfalfa hay was supplied instead of wheat hay, grew apparently at the same rate, even though the A vitamin factor was probably present in more abundance in the alfalfa. Even when animals were kept on oats, barley, and water, alone, the addition of 1.5 cc. orange juice daily protected them from scurvy for at least 80 days, but they did not gain in weight as did the animals supplied with an abundance of hay.

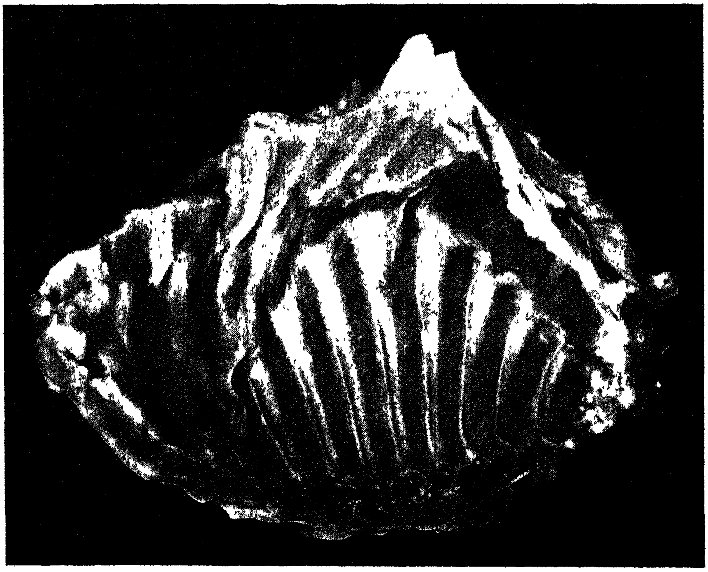

Fig. 9. Section of the ribs showing costochrondral junctions, from guinea-pig fed daily doses of 1 gram of desiccated orange juice. Compare with figure 8.

3. *Concentrated Whole Orange Juice.*—This sample of concentrated orange juice, designated as No. 3, was made from the juice of

very ripe navel oranges. The raw juice was concentrated in a glass enameled vacuum pan under high vacuum, the temperature not exceeding 45° C. at any time, and being for the greater part of the time at or below 40° C. The time required for the processing was about four hours. At the end of the concentration, the sample used in this test was drawn directly from the vacuum pan into one gallon sterilized glass jars stoppered with a sterilized cork and kept moderately cool.

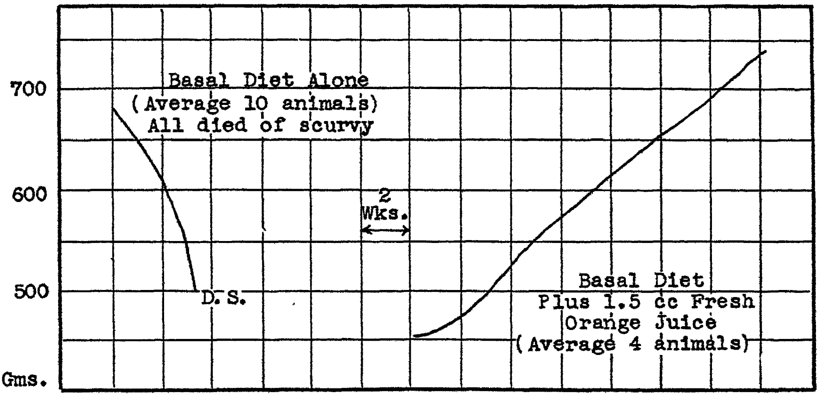

Fig. 10. Growth curves for basal diet alone and for basal diet plus 1.5 cc. fresh orange juice. D.S., died of scurvy.

Because of the unusually high percentage of solids in the raw juice (17 per cent) one gallon of concentrate represented only 5½ gallons of raw juice, while in commercial practice the normal volume concentration is about seven to one. On a weight basis, 1 gram of this concentrate is equivalent to about 4.5 grams of raw juice.

Successively reduced amounts of concentrate beginning with 1 gram and ending with 0.25 gram were fed to normal test animals, each day, together with the basal diet described above. The weights of the animals were recorded and observations made daily for the first appearance of the usually noted symptoms of scurvy.

Guinea-pigs nos. 59, 60, and 61 (fig. 11) were fed on the basal diet plus 1 gram concentrate daily diluted with water (period A to B). At the end of 40 days the dose was reduced to 0.5 gram daily. Animal 59 died after 95 days; no scurvy symptoms were found on post mortem. Animals 60 and 61 showed no signs of scurvy after 115 days. It will be recalled here that on the basal diet alone symptoms were apparent after 20 days, followed by death 10 to 15 days later (fig. 10). A second trial with five younger animals (fig. 11) produced no symptoms in 60 days on a 0.5 gram dose.

A third trial using 0.25 gram doses with three animals was started (fig. 11). One guinea--pig, no. 107 (not plotted in figure) died after 10 days from unknown causes. Animal no. 106 was continued on this dose for 88 days, when it was doubtful if it would continue to live longer. The dose was then increased to 5 grams of the concentrate, partly neutralized with alkali. This increase caused a rapid recovery to normal, although little increase in weight was noticed. This experiment was discontinued on the 115th day, and the animal was placed on greens to recover. Guinea-pig no. 108 gained weight

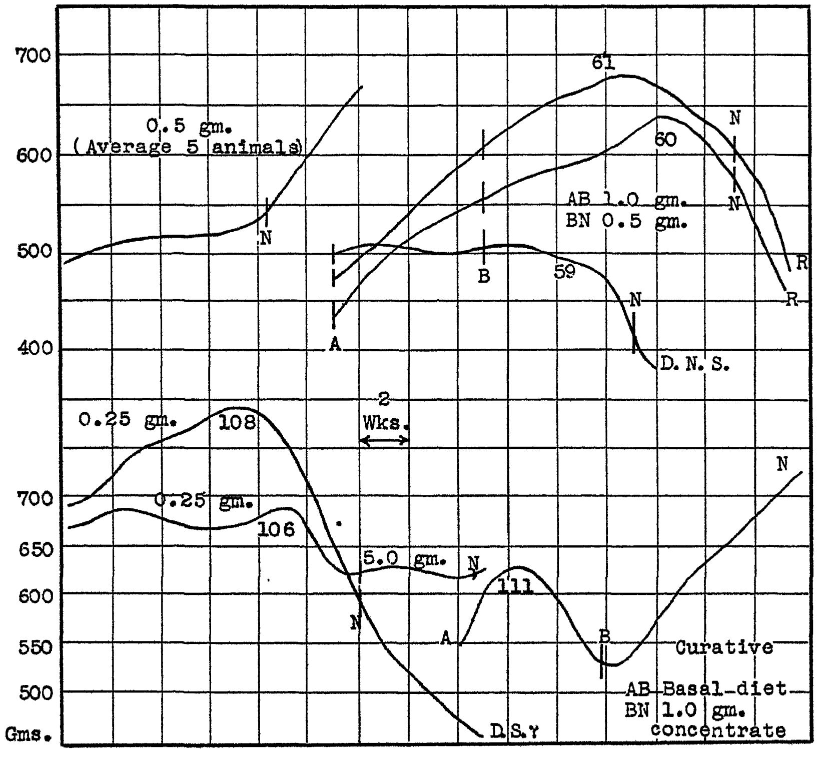

Fig. 11. Growth curves with basal diet plus concentrated whole orange juice (Orange concentrate No. 3). At N fresh grass was given. D.N.S., died, no scurvy found. D.S., died of scurvy. R., recovered.

for 45 days, then lost weight and vitality and appeared scorbutic on the 68th day. After 87 days the diet was changed to normal. Death occurred later, but no typical lesions of scurvy were noted on post mortem examination.

One curative experiment was carried out, using guinea-pig no. 111. This animal was kept on the basal diet until definite symptoms of scurvy were produced. A curative dose of 1 gram of the concentrated whole orange juice, partly neutralized by sodium hydroxid solution at the time of feeding caused the symptoms to disappear in 24 hours and the animal soon recovered to normal.

The above tests indicate that 0.5 gram of the concentrate representing about 2.2 cc. of fresh orange juice was sufficient to protect against scurvy, while 0.25 gram of concentrate, representing 1.1 cc. of fresh orange juice, although delaying the onset of the disease, was not sufficient to protect. Since 1.5 cc. of the fresh orange juice is considered necessary to protect animals from scurvy for at least 90 days, it was concluded that orange concentrate No. 3 had lost little or none of its antiscorbutic properties during concentration and subsequent storage.

4. *Clarified Orange Syrup.*—This clarified syrup product, Concentrate No. 6, was made from whole ripe fruit of miscellaneous varieties with a small amount of added cane sugar, according to the following method: The raw juice was heated in 30 minutes to a temperature of 185° F., and then transferred from the heating kettle to a large wooden mixing tank. In this operation the temperature dropped from 185° F to 150° F. in two hours. About 4½ hours were consumed in filtering the juice, with the aid of filtercel, the juice cooling somewhat during this time. It then stood overnight in an open tank loosely covered with canvas. The total exposure before concentration was about 18 hours, during the first 2½ hours of which the temperature fell from 185° F. to 150° F., during the next 4½ hours from 150° F. to 120° F., and then for a period of about 11 hours while the juice was exposed to the air it fell from 120° F. to about 90° F.

Sufficient sugar was added to make the ratio of solids to acid 12 to 1, and the filtered, sweetened juice was then concentrated in a glass enameled vacuum pan in about 7 hours. The temperature during this time was about 95° F. and did not exceed 100° F. except possibly through slight local overheating, which the thermometer would not indicate. However, as this juice was continually under vacuum of about 28 inches of mercury and under violent agitation while boiling under this vacuum, the chances for local overheating were very slight. The final product contained 72 per cent solids. One gallon of the concentrate represents 5 gallons of the raw juice. One gram of this concentrated syrup represents 3.6 cc. of orange juice.

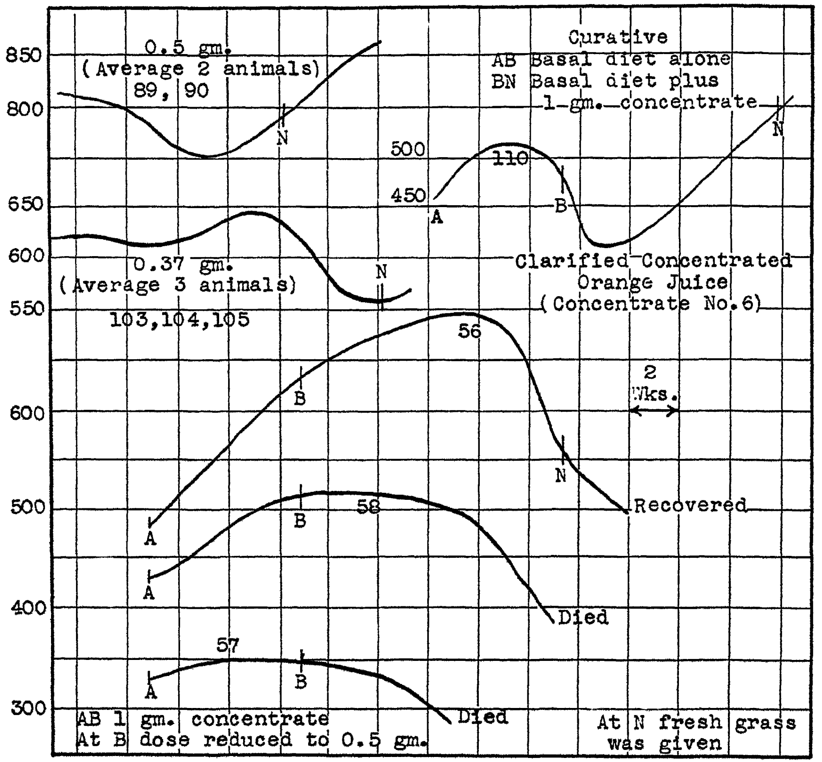

Fig. 12. Clarified concentrated orange juice (Orange concentrate No. 6).

Again, as in the previous trials, successively smaller amounts of the product were administered until the minimum amount which seemed to protect the animals from scurvy was determined.

In a preliminary trial with this concentrate, three guinea-pigs, nos. 56, 57, and 58 (fig. 12), were started on the basal diet plus 1 gram of this product daily. No sign of scurvy appeared in 43 days so the dose was reduced to 0.5 gram of the concentrate, an amount representing about 1.8 cc. fresh orange juice. One animal, no. 57, soon began to fall off in weight and died at the end of 37 days on the reduced amount of juice or a total time of 80 days on the test. Post mortem did not reveal any significant lesions. The test was continued with the other two animals, nos. 56 and 58, and although both began losing vitality, no recognizable symptoms of scurvy developed. Animal 58, on the dose of 0.5 gram concentrate, died after 70 days, or a total of 113 days. Again, on post mortem examination this animal showed no scurvy lesions. The third guinea-pig, no. 56, was placed on grass after it had been on the reduced dose for 75 days or a total of 118 days. It slowly improved in appearance and the test was discontinued.

The experiment using 0.5 gram dose of this concentrate was repeated, using guinea-pigs nos. 89 and 90. No symptoms developed in 60 days.

In a third series of tests the dose of this concentrate was reduced to 0.37 gram, an amount equivalent to about 1.4 cc. of fresh orange juice. Three guinea-pigs, nos. 103, 104, and 105, received this diet.

These animals maintained their weight for 60 days, then began to lose until the 90th day of the experiment, when they were placed on grass to recover. No symptoms of scurvy were noticed at any time. Two of the animals recovered their weight while the third died some time later, revealing no lesions of scurvy at autopsy.

One curative experiment was carried out, using guinea-pig no. 110. The animal was kept on the basal diet until definite symptoms of scurvy appeared. A dose of 1 gram of the concentrated clarified juice administered daily caused the symptoms to disappear rapidly and the animal soon recovered to normal.

In this case the minimum for protection appeared to be between 0.37 and 0.50 gram representing, respectively, 1.3 to 1.8 cc. of fresh orange juice. Although this juice was exposed to much higher temperatures for a longer time, at these temperatures there does not seem to be an appreciable destruction of the vitamin C factor.

5. *Desiccated Orange Juice.*—This product was made in New York in 1922 by a spray drying process and when the experiments began was over two years old. The Exchange Research Laboratory, which furnished the product, reported that cane sugar had been added before the drying, for otherwise a sticky product would have been obtained.

Because of this addition of sugar, 1 gram of the dried product represented only about 3 cc. of fresh orange juice. Accordingly, 0.5 gram of this product, representing 1.5 cc. of fresh orange juice, should protect guinea-pigs from scurvy, provided the C factor was not lowered in drying and subsequent storage.

The product, which was very hygroscopic, was kept in an airtight container and a small amount was taken each day, diluted with water, and the required quantity administered by mouth, just as were the orange juices.

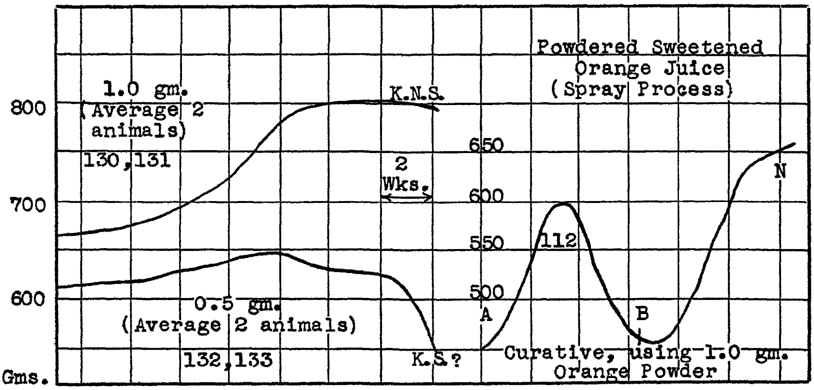

Fig. 13. Experiments with powdered, sweetened orange juice, prepared by a spray process. This desiccated orange juice was two years old at the beginning of the experiment. At N, fresh grass was given. A to B, basal diet alone. B to N, 1.0 gm. powdered orange juice given daily. K.N.S., killed, no scurvy found. K.S.?, killed, scurvy doubtful.

A curative experiment was first tried, using guinea-pig no. 112 (fig. 13). This animal was rendered scorbutic on the basal diet alone and when typical symptoms of scurvy appeared, characterized in this case by painful limbs and face-on-one-side position, 1 gram of the desiccated orange juice was given. The outward symptoms disappeared in less than 12 hours. This animal slowly gained weight and on the 84th day seemed to be fully recovered and was placed on the normal diet.

A second series of tests were carried out, using 1 gram and 0.5 gram of the dry orange juice as a preventive of scurvy. On a 1 gram dose guinea-pigs nos. 130 and 131 (fig. 13) gained weight and appeared to be in perfect health even at the end of the 103rd day. These two animals were killed after the 107th day and a very careful post mortem examination was made to determine if any symptoms of scurvy could be detected. Both animals appeared to be normal in every respect.

Two other guinea-pigs, nos. 132 and 133, fed on 0.5 gram of the powdered orange juice and the basal diet failed to gain in weight, but no symptoms of scurvy were noticeable in 90 days. After 105 days the animals appeared to be weakening and it was noticed that they hopped around the cages as scorbutic animals were previously noted to do. Both animals were chloroformed and only a mild case of scurvy found in one animal at autopsy.

Unfortunately our remaining supply of the dried orange juice accidentally absorbed water and no further experiments were carried on.

Cavanaugh, Dutcher, and Hall have just published a paper[8] in which they include the results of tests with powdered orange and lemon juices made by a spray process similar to that used in making powdered milk. They have concluded also that the antiscorbutic potency of these powdered fruit juices was retained.

Our above limited number of experiments are in agreement with this report and suggest that it is feasible to prepare a desiccated orange juice which retains an appreciable proportion of its antiscorbutic value. However, from a practical standpoint, not much is gained if so much sugar must be added to the juice that the final product represents a concentration of only one to three. A much higher concentration prevails in both of the concentrated orange juices discussed above.

6. *Dried Whole Orange.*—Another product of considerable interest studied was the dry ground whole orange, furnished by the Laboratory of Fruit and Vegetable Chemistry, U. S. Department of Agriculture.

This product was made from cull oranges from which all unsound fruit had been removed. After quartering, the fruit was coarsely ground in a power mill and allowed to stand overnight so that the juice which might have been squeezed out was reabsorbed in the pulp. The ground fruit was then dried on trays in a dehydrator at a temperature of 155°–175° F. for about 8 hours at a fairly low humidity. The moisture was thus reduced from about 80 per cent to about 8 per cent. One part of the dried product represents four to five parts of the original fresh orange. The product has been used as an ingredient in the manufacture of marmalade and mince meat.

In preliminary experiments, 1 and 2 gram doses were found to be sufficiently large to protect the animal from scurvy for at least 90 days (fig. 14, animals nos. 40, 65).

Considerable difficulty was experienced in feeding with a definite small amount of this product which could be considered representative, as it was not found possible to grind the material to a fine powder. The method finally adopted was to crumble the coarsely ground product in a food chopper and stuff the crumbs into small gelatine capsules. Each capsule held approximately 0.2 gram. A definite quantity could thus be administered by mouth each day.

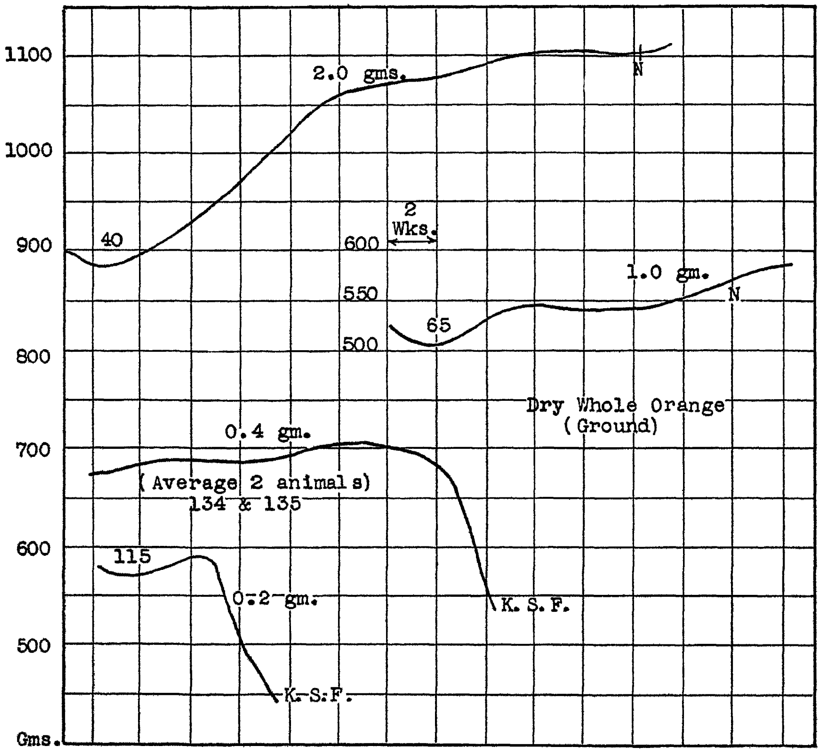

Fig 14. Growth curves with basal diet plus dry whole orange. At N fresh grass was given. K.S.F., killed, scurvy found on post mortem.

In a second series of tests the dose was reduced to 0.4 gram daily. Guinea-pigs nos. 134 and 135 were maintained for 118 days on this amount but after chloroforming the animals it was found on post mortem that definite scurvy symptoms were present. In one case, no. 134, the stomach contained numerous well developed hemorrhages throughout, 2 to 3 mm. in cross-section. On post mortem of no. 135 similar well marked lesions were revealed. There were numerous small hemorrhages in the costochondral muscles. Both fore and hind legs showed unmistakable lesions of scurvy. Beginning about one-third way upon the humerus and extending down to the digits, there was a diffuse hemorrhagic area underneath the facia and between the muscles. The muscles of the right hind leg were spotted with many fair sized diffused hemorrhages extending from the hip joint all the way down to the digits. In this case the muscle proper was apparently involved.

On a 0.2 gram dose one animal, no. 115, lived 52 days but at the end of this time was in a very serious condition, having lost a great deal of its weight. It was then chloroformed and a post mortem examination made. Well developed hemorrhages were found in the large intestine and also underneath the skin in the region of all four legs.

So far as we are aware no information is available which would tell us what is the minimum dose of fresh whole orange which would protect the guinea-pig from scurvy. The therapeutic value of this fruit has generally been believed to be contained in the juice and there is little evidence, we believe, to show that the peel and pulp contain the antiscorbutic factor. However, it is apparent from the above test with 0.4 gram of the dried product, representing about 2 grams of fresh whole orange, that the minimum must be near 2 grams. These few tests indicate that a dry whole orange product may be prepared which furnishes a concentrated source of the antiscorbutic factor.

The question of stability of this factor is next to be considered. The above tests were carried out on material which was over a year old, kept sealed in two-quart fruit jars. Further experiments will be carried out on this material when it has been stored for a much longer time.

7. *Concentrated Lemon Juice.*—This product was produced in the glass enameled vacuum pan, just as was the orange juice No. 3. However, the raw lemon juice was held for 24 hours preserved with 6 ounces of potassium metabisulphite to 100 gallons before being concentrated. Sufficient cane sugar was added to the raw juice to make the ratio of total solids to acid three to one.

Concentration required about four hours, temperature conditions being the same as those prevailing during the preparation of the orange concentrate No. 3. One gallon of the concentrated lemon juice represents 5.9 gallons of the raw juice. One gram of the concentrated lemon juice was equivalent to 4.53 grams of the raw juice.

Considerable difficulty was experienced in finding guinea-pigs that would readily submit to feeding with the concentrated lemon juice, even when considerably diluted with water. Two guinea-pigs, however, were maintained for two months on a 0.5 gram dose, an amount equivalent to about 2.2 cc., without visible signs of scurvy.

The high concentration of citric acid in this product undoubtedly is responsible for the aversion of the animals to the lemon juice. An early attempt to prepare a de-citrated lemon juice from the concentrate by removal of the citric acid with calcium carbonate resulted in a product which failed to protect three guinea-pigs from scurvy. This was to be expected since no precautions were taken to exclude air. It has been shown by Zilva[9] that destruction of the antiscorbutic factor is favored by alkalinity and exposure to air. In this connection Zilva[10] has prepared a concentrated de-citrated lemon juice which was active after three months, having been acidified and stored under anaerobic conditions.

Experiments are now in progress in which an attempt will be made, with the knowledge gained from Zilva's work, to determine the antiscorbutic value of this same concentrated lemon juice, which is now over a year old.

The difficulty of feeding the concentrated lemon juice unaltered has greatly handicapped the experimental work on this product. However, the results given indicate that a considerable portion of the antiscorbutic value of the juice has been retained.

SUMMARY

Feeding experiments with guinea-pigs were made to determine whether the antiscorbutic value of commercially concentrated orange juice was lowered during concentration. Fresh orange juice was used as a standard antiscorbutic for control. Similar experiments were made to determine the antiscorbutic value of dried whole orange and desiccated whole orange juice, and a few experiments were made with a commercially prepared concentrated lemon juice. The data obtained show:

1. That commercial orange juice concentrated in vacuum at a low temperature retains practically all of the antiscorbutic value of the original orange juice.

2. That a clarified concentrated orange juice retained the antiscorbutic factor to a great extent although there is a probability that the longer exposure to the air at higher temperatures lowered this value.

3. That a desiccated orange juice prepared by a spray drying process retained a good proportion of the antiscorbutic value of the fresh orange juice even after two years' storage.

4. That a dried whole orange product prepared in a commercial dehydrator was a very concentrated source of the antiscorbutic factor.

5. That concentrated lemon juice, commercially prepared by evaporation in vacuum at a low temperature, could be considered a concentrated source of the antiscorbutic substance.

ACKNOWLEDGMENTS

The writer wishes to thank C. M. Carpenter, formerly of the Division of Veterinary Science, and J. Traum, a member of the same division, for assistance with autopsies, and also to acknowledge the advice and encouragement of Professor M. E. Jaffa.

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FACTORS AFFECTING EFFICIENCY IN FUMIGATION WITH HYDROCYANIC ACID*

BY

HUGH KNIGHT

INTRODUCTION

In most of the previous efforts to determine gas concentration, rate of diffusion, leakage, etc., in fumigation with hydrocyanic acid the results have been expressed in terms of insect kill. This method is not entirely adequate inasmuch as it fails to provide any data as to the actual concentration of gas present under the tent at any given time during the exposure, the rate of leakage through the tent, or the effect of temperature.

In an effort to gain a more complete understanding of what actually occurs during fumigation the writer has attacked the problem from both the chemical and the entomological standpoint, with the following aims: (1) To ascertain the actual gas concentration necessary to kill coccinellid beetles (*Hippodamia convergens* Guer.) and red scale (*Chrysomphalus aurantii* Mask.); (2) to establish a standard of measurement by which the relative efficiency of different methods of fumigation might be determined; and (3) to apply the results thus obtained to the study of certain factors affecting killing efficiency under the conditions of orchard fumigation.

CONCENTRATION AND TIME FACTORS IN FUMIGATORIUM TESTS

The initial tests under sections (1) and (2) were conducted in a gas-tight fumigatorium with a volume of 100 cubic feet. A glass tube entered from one side and extended to a point somewhat above and at one side of the center. On the outside this tube was inserted into a bottle containing a solution of sodium hydroxide and herein referred to as the aspirator bottle. Gas was drawn through this solution by means of a water-displacement system consisting of two containers, each of 3 liters capacity. The hydrocyanic acid gas, drawn into the aspirator bottle, was absorbed in the sodium hydroxide solution, which was later titrated with a solution of 0.1 N silver nitrate and the concentration of HCN determined. Coccinellid beetles in small containers and scale infested lemons in cheesecloth bags were suspended at a point opposite the intake of the glass tube.

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* Paper No. 122, University of California, Graduate School of Tropical Agriculutre and Citrus Experiment Station, Riverside, California.

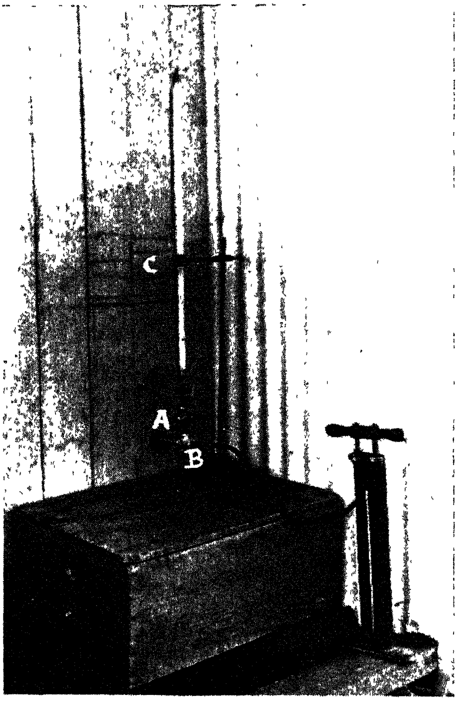

Fig. 1. Hydrocyanic acid was poured into burette, *C*, accurately measured and run into feed pipe through valve *A*, then forced through atomizing nozzle inside box by means of hand pump.

Liquid HCN was atomized in the closed box by means of a piece of ¼-inch pipe fitted with a regular atomizing nozzle. On the outside, the pipe was fitted with a T, to the upright part of which a shut-off valve was attached (*A*, fig. 1). The horizontal outlet to the T was fitted with a piece of 1¼-inch pipe to the end of which a valve stem from an automobile inner tube (with the base sawn off) had been soldered. This allowed an air pump to be securely fastened in place (*B*, fig. 1).

A burette was placed over the shut-off valve *A* (*C*, fig. 1). Liquid HCN was poured into the burette, accurately measured and run into valve *A*; the valve was then closed and the operation of the hand pump forced the liquid through the atomizing nozzle into the tight chamber.

Three liters of gas was drawn through the sodium hydroxide solution at intervals of 2, 5, 10, 20, and 40 minutes, a new bottle being substituted at each interval.

For the first series of tests 100 per cent dosage was used; that is, 20 cc. of liquid HCN (equivalent to 1 oz. of sodium cyanide) per 100 cubic feet under a canvas tent covering a tree of medium size.[1]

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[1] The term "100 per cent dosage schedule" has reference to a particular dosage schedule used in citrus fumigation in California. It was formerly thought that this schedule was as high as could be used with safety to the tree. Designation of the schedule in terms of percentage is desirable because it explains just how much one schedule varies from another; and the 100 per cent or full schedule is taken as the standard. The amount of sodium cyanide (51–52 per cent cyanogen) formerly used in the 100 per cent schedule was approximately 1 oz. to 100 cubic feet for medium-sized trees requiring a dosage of 8 to 10 ozs. On account of the relatively smaller leakage of gas through the tent covering a large and that covering a small tree, due to the difference in ratio of tent area to volume, a small tree under this same schedule requires more and a large tree less than 1 oz. to 100 cu. ft.

When liquid HCN came into use it was necessary to determine just how much by volume would correspond in results on the insects to 1 oz. of the sodium cyanide. Quayle (Cal. Exp. Sta. Bull. 308, June 1919, p. 406) determined this to be 20 cc.; that is, 20 cc. of 96–98 per cent liquid HCN at 60° F. corresponds to 1 oz. of NaCN (51–52 per cent cyanogen). This was determined on the basis of field and laboratory tests on scale and other insects. It also corresponds with the chemical determinations. Gray (Cal. Exp. Sta. Bull. 308, June, 1919, p. 412) has shown that a 90 per cent recovery from 200 lbs. NaCN (52 per cent cyanogen) yields 16.98 gallons of liquid HCN (97 per cent) at 60° F. On this basis 20 cc. of liquid HCN is the equivalent of 1 oz. of NaCN. On the basis of a 95 per cent recovery 200 lbs. NaCN yields 17.93 gals. of liquid HCN, which is equivalent to 21 cc. of liquid HCN to 1 oz. of NaCN. Under the old methods of generation from NaCN in the field the yield of HCN gas was about 90 per cent, with a maximum under the most favorable conditions of 95 per cent.

Woglum (Jour. Econ. Ent., Oct., 1919, p. 360) concluded from tests in the field that 18 cc. of liquid was equivalent to 1 oz. of NaCN; but Mr. Woglum then proceeded to raise the schedule so as to call for more units of 18 cc. than the old schedules called for ounces of NaCN. The final result, that is the given dosage to the tree, was practically the same, but with less correct equivalents of value, and the introduction of a unit of 18 cc. as the standard, which is much less convenient for the continual calculations necessary than the unit of 20 cc. Since liquid HCN is now used exclusively for citrus fumigation in California it would be preferable to designate dosage schedules in terms of cubic centimeters, as 20 cc. schedule, 16 cc. schedule, etc.

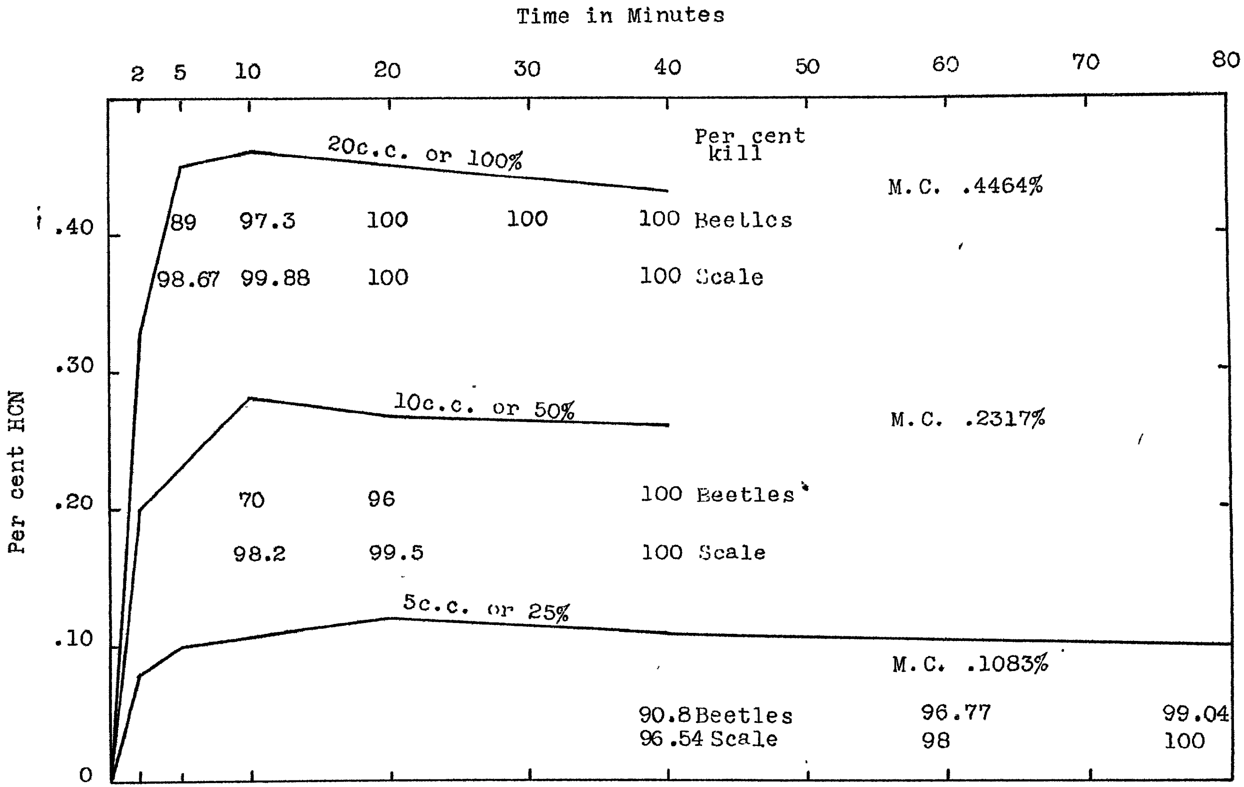

Fig. 2. Graph showing gas concentration in tight fumigatorium corresponding to 100, 50, and 25 per cent dosages respectively, also kill of Coccinellid beetles and red scale at varying intervals of exposure.

NOTE.—The sign % should be added to all figures representing insect kill.

The chart (fig. 2) shows the concentrations of gas reached within the chamber at the intervals stated, also the percentage of both beetles and scale killed. It will be noticed that the concentration increased rapidly for the first 5 minutes, the maximum being reached in 10 minutes, and from that time until the end of the exposure the concentration fell off slowly.

For all practical purposes diffusion under the conditions stated may be said to be complete at the expiration of 5 minutes. A complete kill of both beetles and scale occurred in 20 minutes. As diffusion is not completed until the expiration of 5 minutes, the mean concentration for the period is calculated from that time on according to the formula $\frac{\Sigma \text{MC} \times \text{T}}{\Sigma \text{T}}$ where MC = mean concentration for each time interval, and T = the time interval over which the MC is computed. An average calculated in this way gives due weight to the time element, a proceeding which is essential when calculating concentrations under a tent.

The MC for the 20-minute period was (excluding the first 5 minutes) .448 per cent HCN. This means that for fully 15 minutes the insects had been subjected to an atmosphere of $^{45}/_{100}$ of one per cent HCN, with the result that all were killed. At the end of 5 minutes 89 per cent of the beetles and 98 per cent of the scale were killed; at 10 minutes 97 per cent and 99.88 per cent respectively. The MC for the entire 40-minute period was .446 per cent HCN (excluding the first 5 minutes).

For the second series of tests the dosage was reduced to 10 cc. (50 per cent). The MC for the entire period (excluding the first 5 minutes) was .231 per cent HCN. All the insects were not killed until the full 40-minute period had elapsed. This is particularly interesting as it reaffirms the validity of the fumigation constant (time-concentration factor) explained by Quayle and Knight in 1921.[2] Thus 100 per cent dosage for a period of 20 minutes is equivalent in killing effect to 50 per cent for 40 minutes, or dosage × time = K.[3]

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[2] Quayle, H. J. and Hugh Knight. Fumigation with gas-tight covers, California Citrograph, vol. 6, no. 6, p. 196, April, 1921.

[3] From experiments on black scale eggs in a tight container, Woodworth states, "by doubling the dose we get the same killing effect in approximately a tenth the time." Where the leakage factor enters, as under canvas tents, he states, "a change of one ounce in the dose could be equally well compensated for by a change of 40 per cent in the time. That is, an 8 ounce dose for 45 minutes would have the same killing as a 7 ounce dose for an hour, or a 9 ounce dose for 32 minutes." (C. W. Woodworth, School of Fumigation, p. 173, August, 1915.)

This holds true within certain limits. There is a minimum concentration below which no kill is effected regardless of length of exposure, and vice versa a minimum exposure below which no concentration however high will effect a kill.

For the third series the dosage was again reduced one-half or to 5 cc. (25 per cent). The period of the exposure was doubled, or increased to 80 minutes. The MC was .108 per cent HCN. The beetles were not all killed even after 80 minutes exposure to this concentration, the kill being 99.04 per cent. Scale were all killed at 80 minutes but not at 60 minutes, the kill being 98 per cent at the latter time. At 40 minutes the kill was 90.8 per cent for beetles and 96.54 per cent for scale. The minimum effective concentration evidently had been reached at this point. For the 40-minute interval the kill was far below the minimum necessary for orchard requirements on resistant red scale, and yet most of our commercial fumigation today is below this concentration. The 40-minute interval is taken as an example for the reason that with the present methods practically no gas remains under the tent at the expiration of that time.

The minimum MC or efficiency line lies somewhere between .108 per cent and .231 per cent, or between $^{10}/_{100}$ and $^{23}/_{100}$ of one per cent HCN if 40 minutes be taken as the standard for exposure. This will vary for the same insect in different localities, and for different insects in the same locality. For resistant red scale it is approximately .20 per cent; for black and citricola scale it is approximately .15 per cent, and for coccinellid beetles it is slightly above .20 per cent.

THE STANDARD OF MEASUREMENT OF KILLING EFFICIENCY

From the foregoing facts it may be inferred that the standard of measurement should be the MC-time factor. A further study of the chart (fig. 2) reveals the fact that a heavy dosage diffuses more rapidly than a light one, the peak of the 100 per cent dosage being reached in 10 minutes, while the peak of the 25 per cent dosage was not reached until 20 minutes. It also shows that the light dosage tends to maintain its concentration longer. The slight drop in concentration indicated by the chart may be accounted for in part by leakage around the joints of the fumigatorium since it is impossible to construct a box that is absolutely gas-tight, and in part by the withdrawal of gas at each aspiration, and possibly to a slight extent by adsorption upon the wall surface.

All of the foregoing series of tests were made at temperatures ranging from 60° to 80° F. Figure 3 shows a graph of a test made at 38° F. with 100 per cent dose (20 cc.). At this temperature

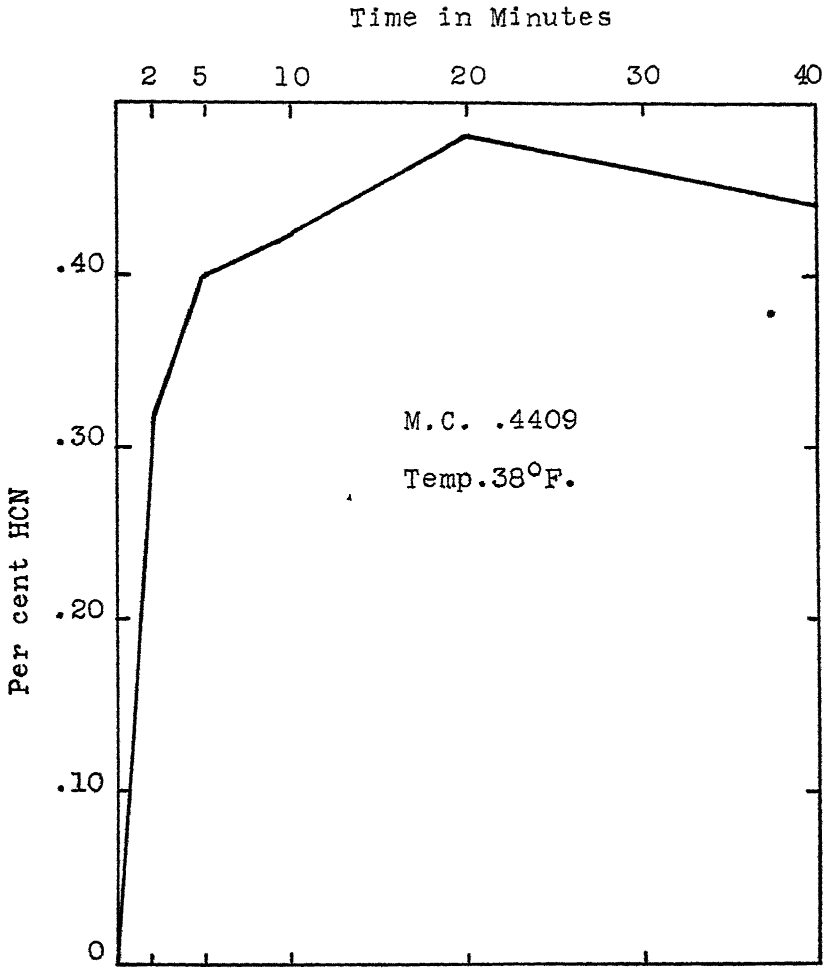

Fig. 3. Test made at 38° F., showing retarded diffusion.

diffusion was considerably retarded, the peak not being reached for 20 minutes. This, however, did not materially affect the MC, which was .440 per cent or practically the same (*allowing for experimental error*) as the other tests at higher temperatures.

TEMPERATURE, CONCENTRATION, AND DIFFUSION UNDER CANVAS TENTS

A great deal has been said about diffusion of gas under the tent; in fact the efforts of the makers of machines for applying HCN seem to be directed entirely to producing more rapid diffusion of gas, instantaneous diffusion apparently being the goal in view. Any gas that is lighter than air will diffuse readily, but if in insufficient concentration it will not kill. It is the concentration of the gas plus its diffusion that kills, not diffusion alone. The kill in any part of a tented area is dependent entirely upon the time-concentration factor. So long as tents or covers are in use that are not gas-tight but permit very rapid leakage, too rapid diffusion must be avoided, for rapid diffusion and rapid leakage go hand in hand, both being dependent upon gas pressure. The pressure of a gas varies with its temperature and with its concentration. The higher the concentration and the higher the temperature the greater the pressure and therefore the more rapid the leakage through the tent. Consequently any attempt to produce a hot gas is a move in the wrong direction, for a hot gas is an active gas under relatively high pressure and diffuses rapidly not only within the tent but through it into the outside air. It follows from all the data so far adduced that the most efficient system of fumigation is that which maintains the highest MC without great variation over the longest time. This is accomplished at the present time by the use of atomized liquid HCN.

In order to determine the effect of temperature on diffusion and concentration of atomized HCN under a tent the following series of tests were made:

An ordinary 8-oz. army duck cover was used over a form measuring 26 × 31 feet. This is an extreme shape and in the grove would come under the class known as "tall trees." This form has a volume of 653 cu. ft., and according to the chart now in use 100 per cent dosage calls for 7 units (of 20 cc.). It will be noted that this dosage per 100 cu. ft. of volume was slightly in excess of that used in the gas-tight chamber. Along the central vertical axis of this form three glass tubes were fastened, each with a single inlet. The inlet of one was a foot from the top; that of the second was at the center, and that of the third was one foot from the bottom. The exits were connected with rubber tubes passing through a short piece of iron pipe buried in the ground, to the aspirator bottles containing sodium hydroxide solution, on the outside, and these in turn were connected with the water-displacement system (fig. 4).

Beetles in small containers and lemons infested with red scale were placed in wire baskets so fastened as to correspond to the height of the aspirator-tube inlets. A range of temperature was chosen from 40° F. to 90° F. so as to approximate the conditions of temperature under which commercial fumigation is practiced. This range was divided into classes as follows: 41°–50°, 51°–60°, 61°–70°, 71°–80°, 81°–90° F. Each class was given four tests and the results are shown graphically in figures 5 and 6. Figure 5 represents the average of four tests made in temperature class 51°–60° F. and may be taken as representative within the temperature range indicated. The letters *T*, *C*, and *B* stand for top, center, and bottom, respectively. It will be noted that at 2 minutes there is an abnormally high concentration at the bottom. The concentration at the top and center continues to rise until the 5-minute interval has elapsed as in the box. At this time diffusion is complete as the concentration at the bottom has fallen and is only slightly (.04 per cent) above that at the top. From this time on the concentration falls rapidly and uniformly until at 40 minutes only .04 per cent remains.

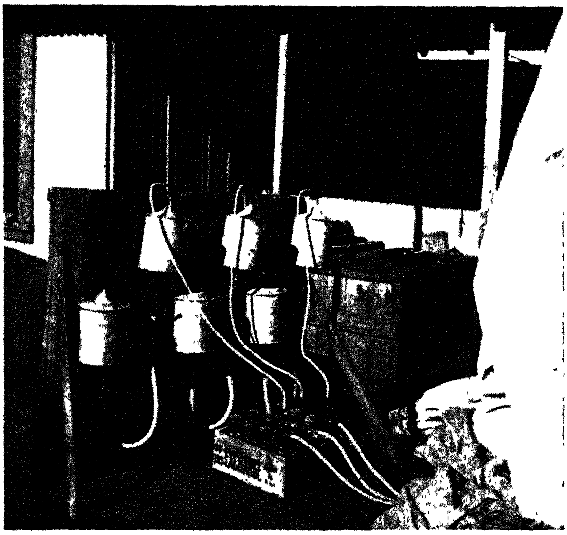

Fig. 4. Arrangement of a battery of aspirators for determining gas concentration at top, center, and bottom of tent.

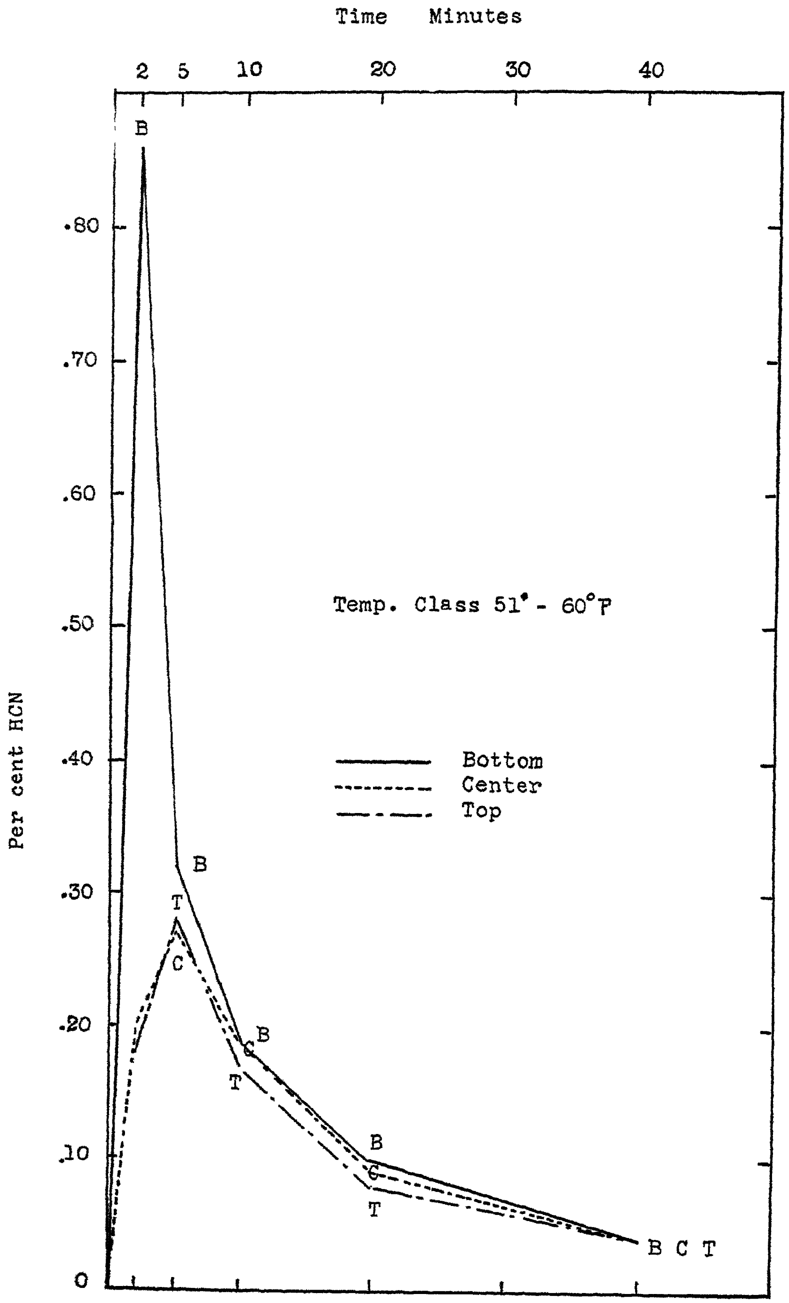

Fig. 5. Gas concentration; average of four typical tests under canvas tents, in temperature class 51°–60° F. *T C B* = top, center, bottom.

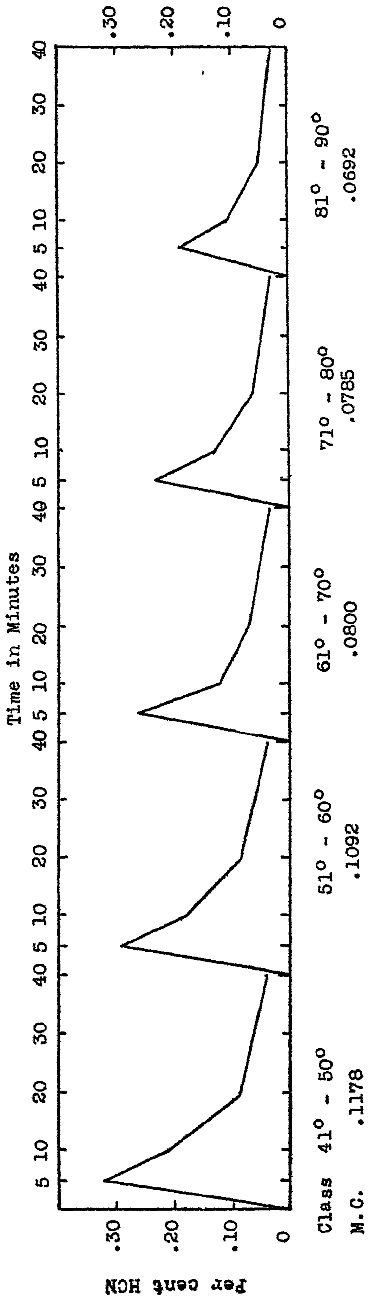

Fig. 6. The effect of temperature on concentration.

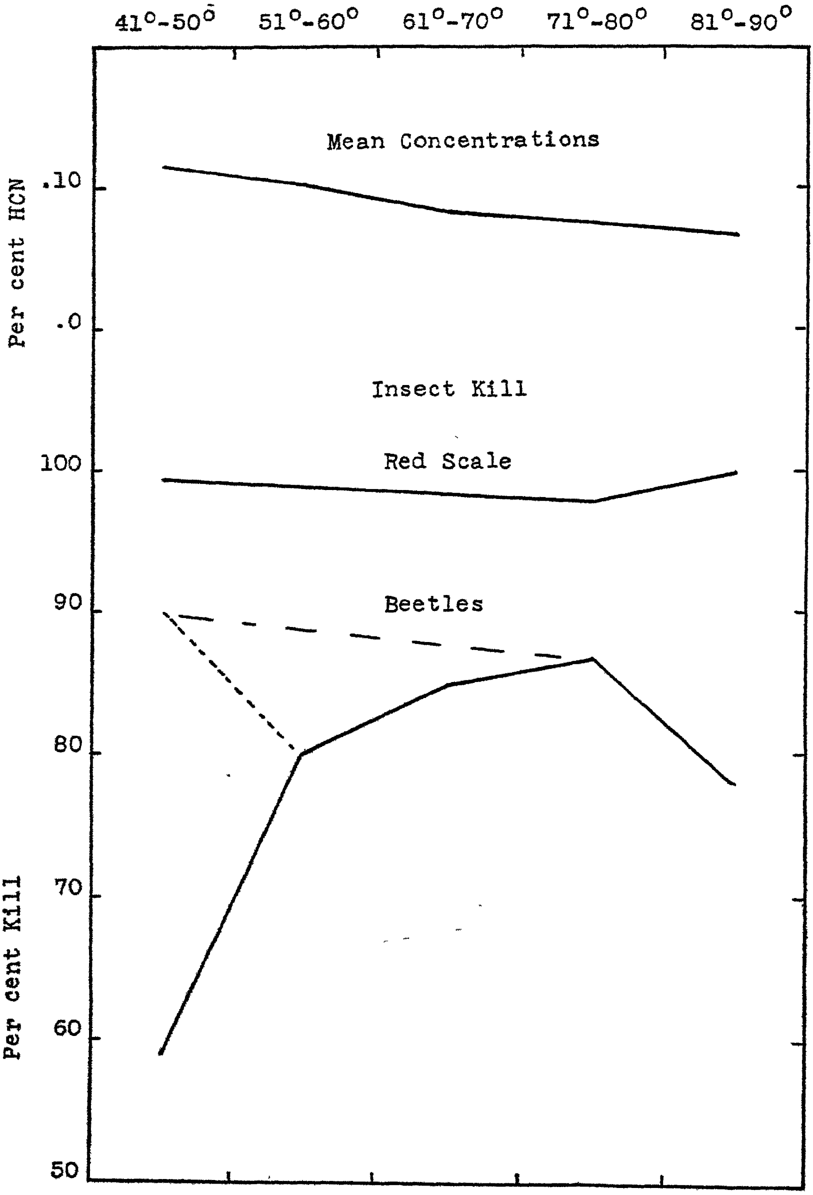

Fig. 7. The relation of temperature to mean concentration and to insect kill.

The mean concentrations for this series were computed on the same basis as those in the tight chamber, that is, from the time diffusion was complete, at 5 minutes. They are given in figures 6 and 7, together with insect kill. A study of these graphs shows that the MC varied in inverse ratio to the temperature. That is to say, the highest concentration is maintained at the lowest temperature, the MC being .117 per cent for the 41°–50° class and .069 per cent for the 81°–90° class. The conclusion is forced upon us that, contrary to popular belief, atomized liquid HCN is more efficient under canvas covers (or any cover with high leakage factor) at low than at high temperatures, and further that even at best the concentration maintained is far below that necessary for a satisfactory kill of resistant red scale and resistant black scale. Figure 7 shows graphically how the MC falls as the temperature rises, and gives the average kill of red scale and beetles. It is interesting to note here that the percentage of red scale killed follows the MC until temperatures between 81°–90° are reached. Up to this point the kill falls with the falling concentration, but near 90° F. the effect of high temperature on the scale itself becomes apparent, resulting in a sharp rise in the kill.

The graph showing kill of beetles is especially interesting. It was known that they were very sensitive to changes of temperature, but it was not realized that their activity was reduced at temperatures above 50° F., so that no precautions were taken to offset this factor until the 41°–50° class was reached, for which the beetles were kept warm (between 70°–80°) up to the time of fumigation. At the same time a check was left with the beetles kept cold in order to gauge the resistance due to inactivity. It will be seen that the point of highest killing efficiency appears to be between 71° and 80° F. As the temperature falls the kill gradually drops to the 51°–60° interval, below that the drop is very abrupt (down to 59 per cent at the 41°–50° interval). The warm beetles on the other hand for the same interval show a sharp rise in kill (99.5 per cent), in fact considerably above the 71°–80° interval, and it is probable that beetles kept at 70° F. would have followed the MC line as indicated by the broken line in graph. Just why the kill of beetles should drop again at the 81°–90° interval (the decrease being principally at the top of the tent), is not so readily apparent. This however is in line with previous experiments, which showed a decrease in kill at the top at temperatures above 80° F. and up to the lethal temperature.

A comparison of the kill between beetles and red scale over the entire series of tests shows the ratio to be as 85 : 100; that is, conditions which killed 85 per cent of the beetles killed 100 per cent of the scale.

Another fact (previously reported by Quayle[4]) that appeared during this series of tests was the large proportion of scale which survived in the moulting stage. Out of 277 live scale found after fumigation, 250 were in the second moult and 27 were adults; that is, 90.25 per cent of the scale found alive were in the moulting stage.

Two tests by the pot method, using 7 ozs. of sodium cyanide, gave a MC of .085 per cent at 56°–59° F. for 40 minutes. Diffusion in this case was complete in 2 minutes. Gas generated by this method is a hot gas (the heat of generation being about 180° F.), and rises to the top of the tent. The tendency to concentrate at the top is reflected in the kill, which was 94.39 per cent at the top, 88.36 per cent at the center, and 88.23 per cent at the bottom. Because of its heat and consequently greater pressure this gas leaks out of the tent more rapidly than atomized liquid HCN.

Assuming that the ratio of dosage unit to volume of enclosure remains constant, then the gas concentration under the same conditions should also remain constant. Therefore, if one unit of 20 cc. to 100 cu. ft. of volume produces a concentration of .45 per cent in a gas-tight container, 7 units to 700 cu. ft. should produce in such a container the same concentration within a reasonable range of variation. The difference if any would be attributable to experimental error. Taking the box as standard it will be seen that in 10 minutes a concentration of .45 per cent was reached. The volume of the form tent was less than 700 cu. ft., being in fact 653, and as it was given 7 units of 20 cc. the ratio of 1:100 was more than maintained. The difference in gas concentration at any given time between that maintained in the gas-tight container and that in the form tent must be attributed to leakage.

In 10 minutes the average MC for the atomizer (at 51°–60°) was .18 per cent, and for the pot it was .12 per cent. In 20 minutes the atomizer gave .09 per cent and the pot .05 per cent. This means that in 10 minutes the leakage of gas with the atomizer amounted to 61 per cent of the amount discharged, and with the pot to 74 per cent.

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[4] Recent fumigation developments, H. J. Quayle. First Annual Report, Calif. Citrus Institute, June 1, 1920, p. 162.

In 20 minutes the leakage with the atomizer was 81 per cent, and with the pot 90 per cent. These figures indicate the superiority of atomized liquid HCN over a hot gas.

TEMPERATURE AND SCALE RESISTANCE

The effect of temperature on the resistance of coccinellid beetles to HCN, as noted during the series of tests, was so marked that an effort was made to determine to what degree scale insects might also be affected.

Coccinellid beetles, lemons infested with red scale, and potted oleander cuttings infested with black scale were placed in refrigerating chambers, held respectively at 30°, 40°, and 50° F., for intervals of 12, 24, 36, and 48 hours, twelve lots in each chamber. They were then taken out and fumigated, together with checks held at room temperature (70° F.). In addition unfumigated checks were kept in order to determine the natural mortality. Checks held at room temperature and fumigated showed a kill of 87.6 per cent for beetles, 99.50 per cent for red scale, and 99.68 per cent for black scale. The natural mortality of beetles was nil, of red scale 29.5 per cent, and of black scale 11.5 per cent. The low mortality of black scale was due to the fact that the cuttings were all young twigs which had no old scale on them.

Of the three the beetles proved most susceptible to varying temperatures, as far as resistance to HCN is concerned. Only 11.4 per cent were killed by fumigation after 24 hours exposure to 30° F. As 87.6 per cent were killed after exposure to 70° F. this indicates an added resistance of 76.2 per cent. Red scale showed an increased resistance of 14.02 per cent after 12 hours exposure to 30° F., but prolonged exposure to this temperature proved fatal to the scale and after 48 hours the natural mortality had increased to 96.36 per cent. The resistance of black scale was increased 15.36 per cent after 12 hours exposure to 30° F., but this scale showed much higher susceptibility to temperature effects, natural mortality rising to 93.13 per cent after only 24 hours exposure.

Red scale showed no effects from a temperature of 40° F. even after 48 hours exposure, natural mortality and kill at all intervals being hardly affected. Black scale, exposed to a temperature of 40° F.,

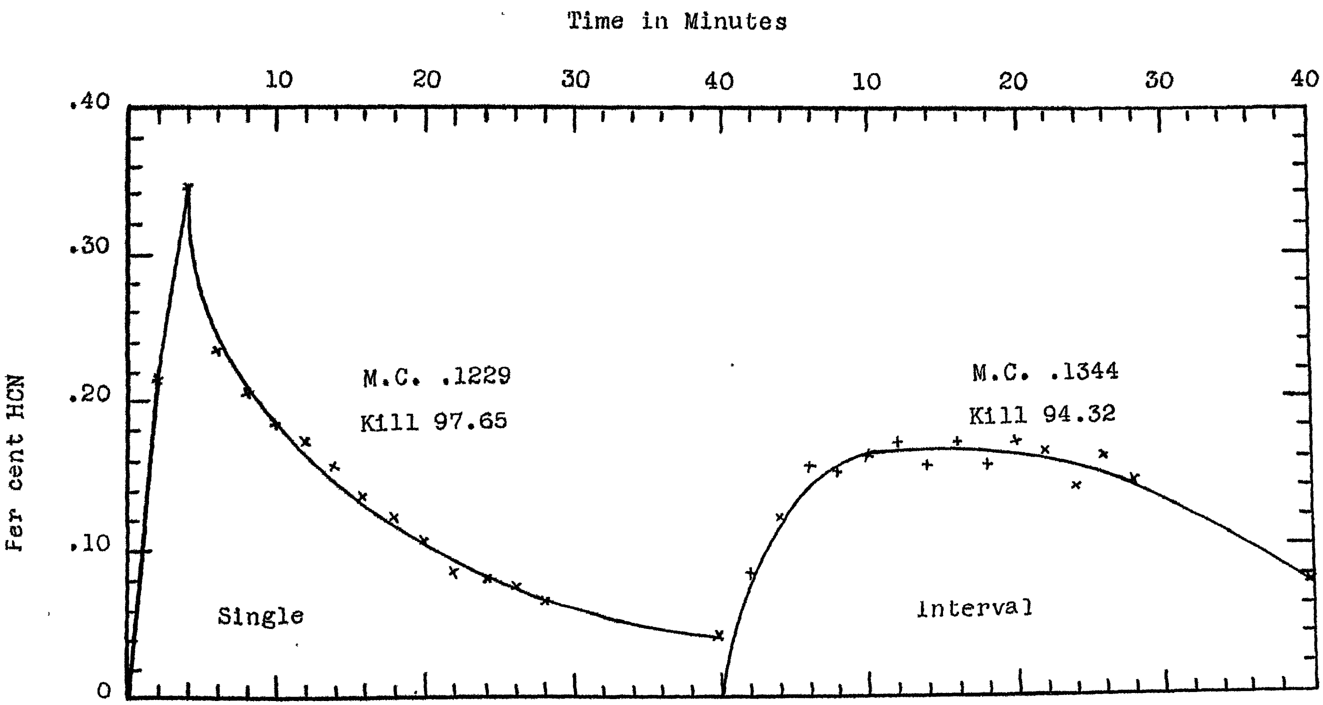

Fig. 8. Comparison of ''single'' and ''interval'' discharge. In the latter, gas was introduced at intervals of 0–2–3–4–5–5–5 minutes, all discharges being equal in amount except the first, which was doubled. Aspiration taken every two minutes for 28 minutes, and a final aspiration made at 40 minutes, the end of exposure.

on the other hand became increasingly resistant to HCN in direct ratio to the length of exposure, the fumigation kill falling from 99.67 per cent to 92.30 per cent at the expiration of 48 hours.

The influence of temperature would seem to vary directly with the activity of the insect; beetles being the most susceptible, black scale much less so, and red scale only slightly.

Resistance of the insect to the effects of HCN is in inverse ratio to its activity. This is true whether inactivity be induced by natural or by artificial means, hence any condition in which the respiratory and metabolic processes are at a low ebb produces increased resistance to fumigation. Insects in the pupal stage and during the moulting period become highly resistant to HCN. The percentage of red scale killed is materially reduced during that period of the year (summer and fall), when reproduction is at its highest and consequently great numbers of scale are in the moult. It has been shown that the resistance of red scale to HCN is not affected by any temperature at which fumigation can be carried on with safety to the tree (from 40° to 85° F.), also that a higher concentration and consequently greater killing efficiency is maintained at low temperatures, and that there are less scale in the resistant or moulting stage during cold weather. For these reasons winter fumigation for red scale is recommended in sections where it has become resistant. An additional argument in favor of fumigation at this time is that the trees themselves are partially dormant and will withstand much higher dosages without injury.

INTERVAL FUMIGATION

The relation of temperature to gas concentration and leakage has been shown. It remains to consider the relation of concentration to leakage.

Other things being equal, gas pressure varies with its concentration. A study of the left-hand graph in figure 8 shows that leakage is most rapid during the period of greatest density or highest concentration. The peak of concentration is reached in 4 minutes. The ensuing 6-minute interval shows a decline in concentration from .35 per cent to .18 per cent or 48.6 of the maximum, the next 10 minutes a decline of 22.8 per cent, and the last 20 minutes one of 17 per cent.

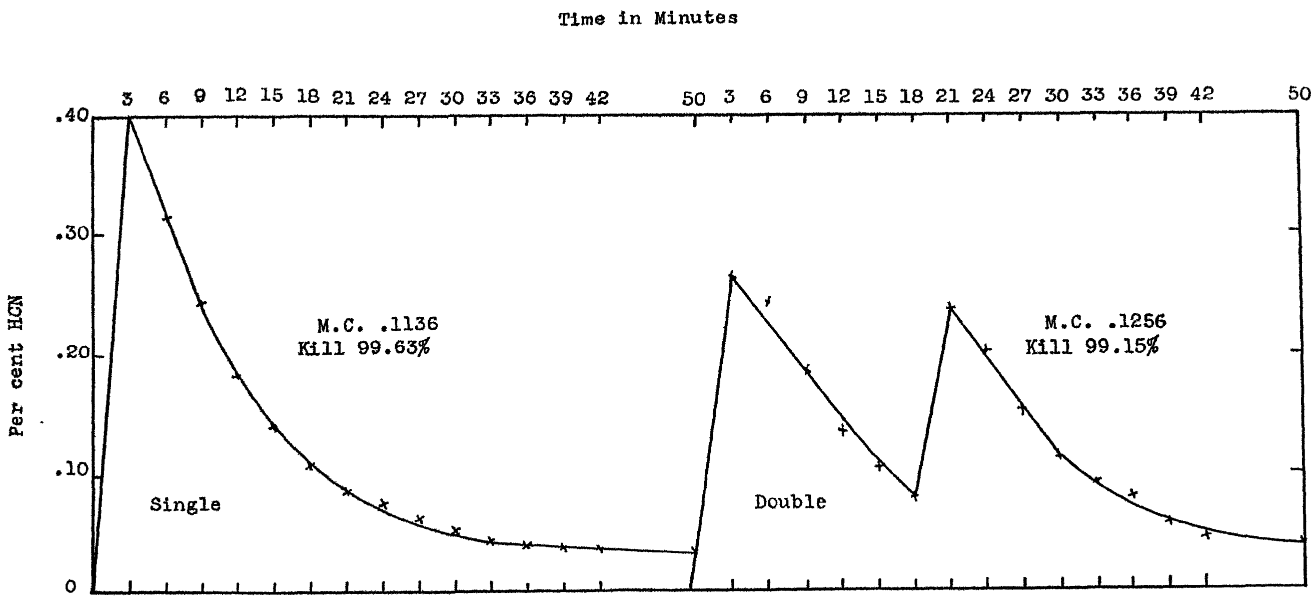

Fig. 9. Comparison of "single" and "double" discharge. In the latter an initial discharge of 62.5 per cent was followed at the expiration of 20 minutes, by a second of 37.5 per cent. Aspirations were made every three minutes for 42 minutes, and a final aspiration at 50 minutes, the end of exposure.

Theoretically, if HCN is introduced into the tent in such a manner as to avoid this high initial concentration, that is, in small units at short intervals, a given quantity of gas should produce a higher mean concentration than with the single-shot method now commonly used.

To test this point a series of experiments was undertaken both with form tents and in the field. Two tents were used and alternated. The same dosage was given in each instance, but in one tent the gas was discharged in the ordinary manner, and in the other at intervals. The gas was aspirated from both tents simultaneously every two minutes until fourteen aspirations had been made; a final aspiration being taken at the expiration of 40 minutes, the end of the exposure.

The graphs (fig. 8) show the results of two field tests. The one on the left represents the ordinary method of application, and that on the right the interval method. In the latter, the gas was introduced a intervals of 0–2–3–4–5–5–5 minutes, all discharges being the same in amount except the first, which was doubled in order to raise the concentration quickly to the killing point. The MC for the two methods was as follows: single, .123 per cent, interval .134 per cent, a difference of .011 per cent in favor of the interval method. This, however, is not reflected in the kill, which was in favor of the single shot by a small margin, being 97.65 per cent for the single shot and 94.32 per cent for the interval method. As it would not be practicable under present fumigation methods to discharge several small units of gas under the tent in the field, a further series of tests was made in which single and double shots were contrasted under identical conditions. This was done under form tents. Each tent received 8 units of 20 cc. liquid HCN, an approximate dosage of 114 per cent (100 per cent dosage being 7 units); in the first tent the entire amount was discharged at once, in the second an initial discharge of 5 units was followed after an interval of 20 minutes by a second discharge of 3 units, or expressed as percentage of the total amount the two discharges were 62.5 per cent and 37.5 per cent respectively. The series comprised six duplicate tests, each including one single and one double shot. In four of the tests aspirations were made, and in all of them lemons infested with red scale were used as a check. The results are graphically shown in figure 9.

As in the previous series the MC is slightly higher for the double shot, being respectively, single .114 per cent, double .126 per cent, but again this is not reflected in the insect kill, which is single 99.63 per cent, double 99.15 per cent (a total of 8600 scales being counted). It is quite probable that when canvas covers with a high leakage factor are used the MC-time factor is slightly modified in practice by a high initial concentration. It is conceivable that between two similar MC's, that produced by an initial high concentration (analogous to a "knockout" blow) might be more effective than that in which the concentration remained fairly constant throughout the exposure.

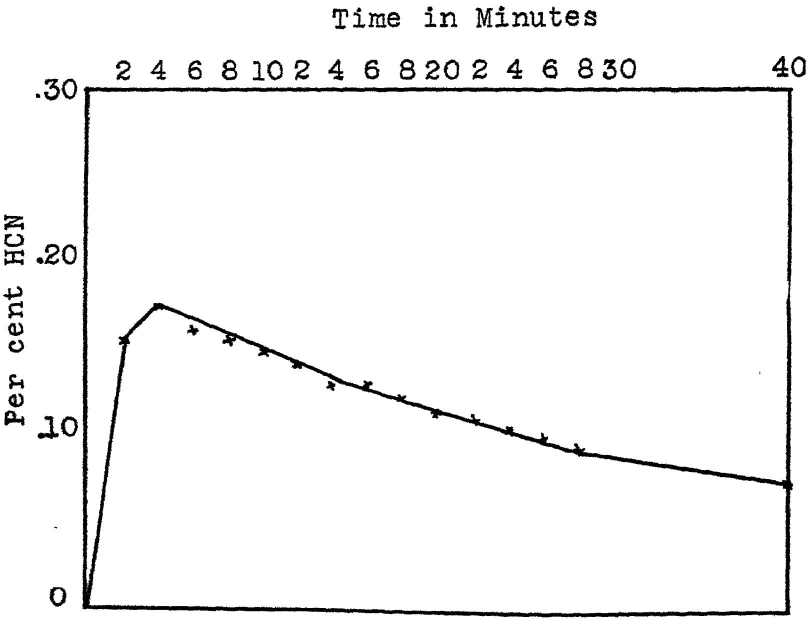

Fig. 10. Curve produced by generation of HCN from calcium cyanide dust. Note similarity to curves in figure 4 in gas-tight container.

At all events the results of these tests indicate that there is no increase of efficiency to be gained by double shooting in the field.[5] Further studies are necessary to determine whether injury to the tree is lessened by avoiding the initial high concentration which occurs in single shooting.

To complete the series, figure 10 shows a curve (average of five tests) produced by generation of HCN from calcium cyanide dust.

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[5] Since going to press other tests have been made in the field, under commercial conditions, the results of which corroborate the conclusions reached above.

As the dosage varies from 100 per cent to 300 per cent the MC has no significance and is not given. The dust was introduced under the tent by means of a hand dusting machine of conventional type, fitted with a large fan-shaped nozzle; this was pointed downward and the dust spread as evenly as possible over the ground surface. When the dust is applied in this manner the danger of injury to the tree is greatly reduced. In order to obtain mean concentrations similar to those produced by liquid HCN a dosage of nearly 150 per cent is required. By referring to figure 2 it will be seen that the curve produced by $Ca(CN)_2$ dust more nearly resembles that produced in a gas-tight container than does any other of this series.

SUMMARY

Aspiration tests conducted in a gas-tight fumigatorium, with coccinellid beetles and red scale used as checks, indicate that it requires a mean concentration of about .45 per cent HCN for 20 minutes to kill every insect.

In a gas-tight container the time and concentration factors may be varied reciprocally within certain limits. That is, if the concentration be reduced the exposure must be increased in the same ratio, or time × concentration = K.

For an exposure of 40 minutes the mean concentration necessary to kill resistant red scale is approximately .20 per cent HCN and for black and citricola scale approximately .15 per cent HCN. In commercial fumigation, the concentration is generally below these amounts.

Leakage is influenced by both concentration and temperature. The highest concentration for a given dosage is maintained at the lowest temperature. The most efficient method of fumigation is by means of atomized liquid HCN.

Insects become resistant to hydrocyanic acid when they become dormant or inactive, whether this condition is brought about by pupation, moulting, or by low temperature. Susceptibility to the effects of temperature varies directly with the activity of the insect. Beetles are more susceptible than scale, and black scale more than red. Red scale does not become resistant to hydrocyanic acid at any temperature at which fumigation can be carried on with safety to the tree.

A series of tests both under form tents and in the field, to determine the relative efficiency of the single-discharge and the interval-discharge methods of fumigation, showed that there is no practical advantage in the interval method so far as scale kill is concerned.

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AN ACCURATE METHOD OF CALCULATING ICE CREAM MIXES

BY

G. D. TURNBOW AND C. M. TITUS

INTRODUCTION

One of the most important factors in the manufacture of ice cream of high quality is the proper proportioning of the ingredients. Formulas are used by a few ice cream manufacturers, but a thorough knowledge of how to standardize the mix (mixture) by using ingredients with varying composition, and thereby bringing about the desired per cent of fat, milk-solids-not-fat, total solids, *et cetera,* is of greater value than the possession of a great number of formulas.

To be able to calculate a mix simply and accurately is very useful to the ice cream manufacturer. Many requests have been received by the Division of Dairy Industry, asking how this can be done. Several methods have been published during the past year or so for computing mixes, but the one presented here seems simpler than any other yet suggested. This method makes use of algebraic formulas, and is more accurate than the "cut and try" method used by many. Rules based on these formulas are given for those who would prefer rules to algebraic formulas.

Composition of Ingredients: Before the composition of a mix can be calculated, a standard must be decided upon. By a standard is meant the per cent of milk-solids-not-fat, sugar, gelatin, and water for gelatin, that the finished mix is to contain. Every ice cream manufacturer has some kind of standard. The sum of the per cents of solids in the ingredients used determines the per cent of total solids in the mix.

The California State law requires 10% fat in plain ice cream. An excellent ice cream can be made by using the following standard, which is the one now used by the University of California:

Fat, 10.5%
Milk-solids-not-fat, 10%
Sugar, 14.5%
Gelatin, .5%
Water, 3% for hydrating gelatin.

Before any standard can be used, its ingredients must, of course, be known. It is convenient to have the ingredients tabulated as in table 1.

Ingredients:

TABLE 1

LIST OF INGREDIENTS FOR REFERENCE AND KEY TO CASES

1. Cream
2. Milk—whole
3. Milk—whole condensed (evaporated milk)
4. Milk—whole condensed and sweetened
5. Skim milk
6. Skim milk condensed
7. Skim milk condensed and sweetened
8. Whole milk powder
9. Skim milk powder
10. Butter
11. Sugar ⎫
12. Gelatin ⎬ all cases
13. Gelatin water ⎭
14. Water

Case I. Ingredients—Cream, milk, condensed skim milk
Case II. Ingredients—*1, 2, 6+3 or 4 or 6
Case III. Ingredients—1, 2, 3
Case IV. Ingredients—1, 2, 4
Case V. Ingredients—1, 5
Case VI. Ingredients—1, 2+condensation
Case VII. Ingredients—1, 5, 6
Case VIII. Ingredients—Left over, 1, 2
Case IX. Ingredients—10, 1, 6
Case X. Given amounts of two or more ingredients
Case XI. Other standards

Cream: Cream is used quite extensively as the main source of fat in ice cream. The more fat it contains, the less of other solids it can have. The fat crowds out the milk-solids-not-fat as shown by the following table:

---

* These numbers refer to the numbers given to the ingredients listed in Table 1.

Milk: Legal milk must contain at least 3 per cent of fat. It must be remembered that milk increases the milk-solids-not-fat about twice as fast as it does the fat. It is almost universally used in building up fat and milk-solids-not-fat in ice cream.

Sweetened Condensed Milk: The constituents of sweetened condensed milk are the same as those of fresh milk, except that they have been concentrated by evaporating in vacuo and have had a varying amount of sucrose, usually about 42%, added to them. This product is used to a considerable extent in the manufacture of ice cream.

Evaporated Milk: Evaporated milk is practically the same product as sweetened condensed milk except that it lacks the addition of the sucrose.

Condensed Skim Milk: It is necessary to build up the milk-solids-not-fat by means of some condensed product; this is usually done with condensed skim. The per cent of solids in the condensed skim milk varies in different plants and varies from day to day in the same plant. Therefore, a very careful check should be kept on the per cent of milk solids in the condensed skim. It usually is the cheapest source of additional milk-solids-not-fat. Probably the most desirable per cent of milk-solids-not-fat in condensed skim is 32.

Skim Milk Powder: Skim milk powder is also used to some extent in localities where it is difficult to secure the condensed skim. The powder makes a very good quality of ice cream, and while it seems perfectly dry to the hand, it contains from two to three per cent moisture. For all practical purposes, it can be considered as containing 97 per cent milk-solids-not-fat.

Whole Milk Powder: Whole milk powder can be purchased from powder plants, but must be used within a short time after manufacture. It is made by powdering the whole milk, and may be used where conditions and price will warrant.

Butter: Sweet butter is used to some extent in the manufacture of ice cream. Sweet butter is that which contains no salt. Since its composition varies, it should be purchased on a fat basis. It usually contains approximately 83 per cent fat. A first class ice cream can be made from it. Even salted butter may be used by melting it at a temperature not exceeding 150° F. and allowing the salt, curd and water to settle out. After they have settled out, they should be drawn off and if necessary, the oil should be washed with warm water. By this process a butter oil practically 100 per cent pure may be secured.

Granulated Sugar: Granulated sugar, either beet or cane, may be used in the manufacture of ice cream, and should be figured as containing 100 per cent solids. Washburn says "that sugar, if stored in a damp place, will absorb only ¼% moisture or less."

Corn Sugar: Corn sugar contains about 10 per cent moisture, is only about 60 per cent as sweet as cane sugar, and may be used to a limited extent to replace granulated sugar.

Gelatin: Gelatin is a very important ingredient in the manufacture of ice cream, but is used only in small amounts, about .5 of one per cent or less. It absorbs moisture very readily. The best grades of gelatin contain from 5 to 10 per cent moisture and none but the best should be used.

Gelatin Water: Some ice cream manufacturers dissolve the gelatin in water at the rate of six parts of water to one of gelatin. It should be dissolved in cold water and heated in a steam jacketed kettle from 165° to 185° F., the temperature varying in different plants. The gelatin solution is added to the mix while it is being held at a pasteurizing temperature.

The Method of Computing the Mix: As suggested above, the method here explained involves the use of algebraic formulas and rules based on these formulas. Thus, there is no "cutting and trying" in it, and the results obtained, when carefully "checked," are bound to be correct—not just rough approximations. For those who know a little elementary algebra, the formulas will be found very simple; a little practice with them will insure both speed and accuracy in computing mixes. Those who have no knowledge whatever of algebra will find it best to use the *rules* at first, but even *they* should compare the rules with the formulas and should study the examples worked out by means of the latter. If they will do this, it is believed that they will soon be able also to use the formulas efficiently and will prefer them to the more cumbersome rules.

Although some examples are worked out showing how to adapt the formulas to other standards, the standard used in this circular is the following:

Fat, 10.5%
Milk-solids-not-fat, 10%
Sugar, 14.5%
Gelatin, .5%
Water, 3% for hydrating gelatin.

Skim Milk: Skim milk may be used in increasing satisfactorily the milk-solids-not-fat.

*TABLE 2

SHOWING PER CENT OF FAT, AND MILK-SOLIDS-NOT-FAT IN MILK AND CREAM

Product	Per cent fat	Per cent milk-solids-not-fat	Product	Per cent fat	Per cent milk-solids-not-fat
Water	0.00	0.00	Cream	30.0	6.24
Skim milk	.02	8.91	Cream	31.0	6.15
Milk	3.0	8.61	Cream	32.0	6.06
Milk	3.5	8.71	Cream	33.0	5.97
Milk	4.0	8.81	Cream	34.0	5.88
Milk	4.5	8.92	Cream	35.0	5.79
Milk	5.0	9.02	Cream	36.0	5.70
Cream	15.0	7.57	Cream	37.0	5.61
Cream	16.0	7.48	Cream	38.0	5.52
Cream	17.0	7.40	Cream	39.0	5.44
Cream	18.0	7.31	Cream	40.0	5.35
Cream	19.0	7.22	Cream	41.0	5.26
Cream	20.0	7.13	Cream	42.0	5.17
Cream	21.0	7.04	Cream	43.0	5.08
Cream	22.0	6.95	Cream	44.0	4.99
Cream	23.0	6.86	Cream	45.0	4.90
Cream	24.0	6.77	Cream	46.0	4.81
Cream	25.0	6.68	Cream	47.0	4.72
Cream	26.0	6.59	Cream	48.0	4.63
Cream	27.0	6.50	Cream	49.0	4.54
Cream	28.0	6.42	Cream	50.0	4.45
Cream	29.0	6.33			

Note: Other products are purchased on known composition.

CASE I.—*Milk, Cream, Condensed Skim.*

Several different combinations of ingredients are used in ice cream, the most common one being composed of milk (or skim milk), cream (or butter) and condensed skim. This combination will be treated first and called case I.

Let M = No. lbs. of mix
a = %[†] fat in milk
b = % fat in cream or butter
c = % [‡]M.S.N.F. (Milk-solids-not-fat) in condensed skim
d = % M.S.N.F. in milk or skim milk
e = % M.S.N.F. in cream

---

* Taken in part from Farrington and Woll.

[†] Thus if 4% milk is used, $a=4$

[‡] Thus if 32% condensed skim is used, $c=32$

The sugar, gelatin and water make up 14.5%+0.5%+3.0% or 18% of the mix.

Hence, 100%−18%=82% (or .82) of the mix consists of dairy products; namely, milk, skim milk, or butter and condensed skim.

Now let X=No. lbs. of a % milk required
Y=No. lbs. of b % cream required
Z=No. lbs. of c % condensed skim required.

Then the formulas are as follows:

$$Z=\frac{10(b-a)+10.5(d-e)-.82(bd-ae)}{c(b-a)-(bd-ae)}\times M.$$

$$Y=\frac{(10.5-.82a)M+aZ}{b-a}.$$

$$X=\frac{10.5M-bY}{a}.$$

It should be remembered that bd means b times d, $bd-ae$ means to subtract a times e from b times d, etc. $10(b-a)$ means to subtract a from b and multiply the difference by 10, etc.

Next, an example is worked out showing how to substitute in the formulas, and then a shorter solution of the same example is given. Afterward the corresponding rules are stated, pages 64 to 66.

Example 1. **(Milk, Cream and Condensed Skim)**

Using standard mix with ingredients having the following compcsition, to make 1000 lbs. of mix:

Milk, 4% fat
Cream, 35% fat
Condensed skim, 32% M.S.N.F.

Thus:

$$a=4$$
$$b=35$$
$$c=32$$
$$\text{From Table}\left\{\begin{array}{l}d=8.8^{*}\\ e=5.8\end{array}\right.$$

---

* Note that d and e are taken to the nearest 10th only, because using the 100ths will never make more than a fraction of a pound difference in the value of Z for a 1000 lb. mix, and furthermore, these per cents are never known exactly for any given composite milk.

Then substituting these numbers in the formula for Z.

$$Z=\frac{10(35-4)+10.5(8.8-5.8)-.82(35\times 8.8-4\times 5.8)}{32(35-4)-(35\times 8.8-4\times 5.8)}\times 1000$$

$$=\frac{10\times 31+10.5\times 3.0-.82(308.0-23.2)}{32\times 31-284.8}\times 1000$$

$$=\frac{310+31.5-233.5}{992-284.8}\times 1000=\frac{108.0}{707.2}\times 1000$$

=152.7 lbs. condensed skim.

Now substituting in the formula for Y, we have

$$Y=\frac{(10.5-.82\times 4)\times 1000+4\times 152.7}{35-4}$$

$$=\frac{7220+610.8}{31}=\frac{7830.8}{31}=252.6\text{ lbs. of cream.}$$

To find X, we have

$$X=\frac{10.5\times 1000-35\times 252.6}{4}$$

$$=\frac{10500-8841}{4}=414.8\text{ lbs. of milk.}$$

Preliminary check:

Total dairy products
=.82×1000 lbs.=820 lbs.

152.7
252.6
414.8
820.1 check.

The complete "check" follows:*

10.5% of 1000 lbs.=105 lbs. of fat required.
In this case there are only two sources of fat, namely, milk and cream.
4% of 414.8 lbs.= 16.59 lbs. fat from milk.
35% of 252.6 lbs.=88.41 lbs. fat from cream.
105.00 lbs. fat altogether which "checks" with the fat required.

The M.S.N.F.=10% of 1000 lbs.=100 lbs. required. There are *three* sources of M.S.N.F. in this case, namely: milk containing 8.8%, cream containing 5.8%, and condensed skim containing 32%.

8.8% of 414.8= 36.50 lbs.=M.S.N.F. from milk.
5.8% of 252.6= 14.65 lbs.=M.S.N.F. from cream.
32% of 152.7= 48.86 lbs.=M.S.N.F. from condensed skim.
100.01 lbs. "Checking" the M.S.N.F.

---

* It must be noted that the preliminary check given above is not a perfect "check" since the amounts obtained for all the different ingredients might be wrong and yet they might total 820 lbs. Hence the more complete check should always be used.

This solution may seem long, because the substitutions have been made at length in the formulas and all the steps in the process shown. After working out a few mixes in this way and being sure of the meaning of the formulas, the shorter form given below should be used:

The formulas or rules should be kept where they may be referred to quickly and the given numbers written down as here shown.

```
                                                         35
                                                        ×8.8
                                                        ----
           To find Z:                                    280
                                                        280              5 8
             M=1000                                     -----       ae=  ×4
              a=4  }                                    308.0           ----
              b=35 } 31=b-a                             -23 2  ............ .23.2
              c=32                      31              -----
              d=8 8 }                   ×10             284.8=bd-ae
              e=5.8 } 3.0=d-e           ---              ×.82
   c(b-a)=31  -------------             310             -----
         ×32  10 5×3=31.5...      .....+31.5            22784 }
        ----        •                 ------              570 } *
       992.0                           341.5            -----  }
 bd-ae=284 8                          -233.5 .........  233.54 }
       -----                          ------
       707 2......................707.2)108.0(.1527 lbs.
            1000×.1527=152.7 lbs. condensed skim or Z.
```

```
   To find Y:
            .82
            ×4
            ---      10.5
     .82a=3.28.......-3.28
                     -----
                       7.22×1000=7220.0
                aZ=    4×152.7  = 610.8
                                  ------
                           31)7830.8(252.6 lbs. cream or Y.
   To find X:
           10.5×1000=10500
           35×252.6 = 8841
                      -----
                   4)1659(414 8 lbs. milk, or X.
```

Hint: Study the short solution given above in connection with the rules.

Rule 1. For finding Z, the amount of condensed skim.

Step 1. Subtract the per cent of fat in the milk from the per cent of fat in the cream: $35-4=31$.

Step 2. Multiply the result of step 1 by the per cent of M.S.N.F. in the mix: $10\times31=310.0$.

Step 3. Subtract the per cent of M.S.N.F. in the cream from the per cent of M.S.N.F. in the milk: $8.8-5.8=3.0$.

---

* Contracted multiplication.

Step 4. Multiply the result of step 3 by the per cent of fat in the mix: 10.5×3.0=*31.5.*

Step 5. Add the results of steps 2 and 4: 310+31.5=*341.5.*

Step 6. Multiply the per cent of M.S.N.F. in the milk by the per cent of fat in the cream: 35.0×8.8=*308.0.*

Step 7. Multiply the per cent of M.S.N.F. in the cream by the per cent of fat in the milk: 4.0×5.8=*23.2.*

Step 8. Subtract the result of step 7 from the result of step 6: 308.0−23.2=*284.8.*

Step 9. Multiply the result of step 8 by the per cent, expressed as a decimal, of dairy products in the mix (for standard used): .82×284.8= *233.5.*

Step 10. Subtract the result of step 9 from the result of step 5: 341.5−233.5=*108.0* which is the numerator of the formula.

Step 11. Multiply the result of step 1 by the per cent of M.S.N.F. in the condensed skim: 32×31= *992.0.*

Step 12. Subtract the result of step 8 from the result of step 11: 992.0−284.8=*707.2.*

Step 13. Divide the result of step 10 by the result of step 12: 108.0÷707.2=*.1527.*

Step 14. Multiply the result of step 13 by the number of pounds in the mix: 1000×.1527=*152.7* lbs. of condensed skim required.

Rule 2. For finding Y, the amount of cream required.

Step 15. Multiply the per cent of fat in the milk by the per cent expressed decimally of dairy products in the mix: .82×4.0=*3.28.*

Step 16. Subtract the result of step 15 from the per cent of fat in the mix: 10.5−3.28=*7.22.*

Step 17. Multiply the result of step 16 by the number of pounds in the mix: 1000×7.22=*7220.*

Step 18. Multiply the result of step 14 (the amount of condensed skim required) by the per cent of fat in the milk: 4.0×152.7=*610.8.*

Step 19. Add the results of steps 18 and 17: 7220.0+610.8=*7830.8.*

Step 20. Divide the result of step 19 by the result of step 1 (Rule 1) 7830.8÷31=*252.6* lbs. of cream required.

Rule 3. For finding X, the amount of milk required.

Step 21. Multiply the per cent of fat in the mix by the number of pounds in the mix: 1000×10.5=*10500.*

Step 22. Multiply the result of step 20 (the amount of cream required) by the per cent of fat in the cream: $35.0 \times 252.6 = 8841.0$.

Step 23. Subtract the result of step 22 from the result of step 21: $10500 - 8841 = 1659$.

Step 24. Divide the result of step 23 by the per cent of fat in the milk: $1659 \div 4.0 = 414.8$ lbs. of milk required.

CHECKS: Check the results in the manner shown on page 63, which is, briefly, as follows:

Find the amount of fat and M.S.N.F. required for the mix, according to standard used. Then find the amount of fat in the results obtained for all three ingredients, condensed skim, cream and milk. These amounts should agree closely with these required for the total mix.

CASE II. *Milk, cream, condensed product:*

This is the most general case treated. Besides the ingredients considered in Case I, it involves the use of plain condensed whole milk or sweetened condensed whole milk or sweetened condensed skim. The letters a, b, d, and e are used with the same significance as they have in Case I.

But since there may now be another source of fat (the condensed whole milk) and another source of sugar (the sweetened condensed, either whole or skim) the formulas must provide for these also. Hence,

Let h = per cent of fat in the plain condensed or sweetened condensed whole milk, if either of these be used. Otherwise, $h = 0$.

Let k = 100 per cent less the per cent of sugar in the sweetened condensed whole or skim, as the case may be. Thus if the sweetened condensed contains 42% sugar, $k = 100\% - 42\%$ or .58.

As in Case I.

X = No. lbs. of a% milk required,
Y = No. lbs. of b% cream or butter required,
Z = No. lbs. of c% condensed product required.

THE GENERAL FORMULAS FOLLOW:

$$Z = \frac{10(b-a) + 10.5(d-e) - .82(bd-ae)}{c(b-a) + h(d-e) - k(bd-ae)} \times M.$$

$$Y = \frac{(10.5 - .82a)M + akZ - hZ}{b-a}.$$

$$X = \frac{10.5M - bY - hZ}{a}.$$

Example 2. **(Sweetened Condensed Skim)**

Using standard mix with ingredients having the following composition and computing a 1000 lb. mix:

Milk, 4%
Cream, 40%
Sweetened condensed skim milk, 32% M.S.N.F.
(Cane) sugar, 42%.

Thus $M=1000$
$a=4$
$b=40$
$c=32$
$d=8.6$
$e=5.4$
$h=0$ (since the fat in the skim milk is practically Zero)
$k=.58$ $(=100\%-42\%)$

Computing Z first:

$$Z=\frac{10(40-4)+10.5(8\ 6-5\ 4)-.82(40\times 8.6-4\times 5.4)}{32(40-4)+0(8\ 6-5.4)-.58(40\times 8.6-4\times 5.4)}\times 1000$$

$$=\frac{10\times 36+10.5\times 3.2-.82\times 322.4}{32\times 36+0-.58\times 322.4}\times 1000$$

$$=\frac{360+33.6-264.4}{1152-187}\times 1000=\frac{129200}{965}=133\ 9 \text{ lbs. of sweetened condensed skim.}$$

$$Y=\frac{(10.5-.82\times 4)\times 1000+4\times .58\times 133.9-0\times 133.9}{40-4}$$

$$=\frac{7220+310\ 6-0}{36}=\frac{7530.6}{36}=209.2 \text{ lbs. cream.}$$

$$X=\frac{10500-8368-0}{4}=\frac{2132}{4}=533.0 \text{ lbs. milk.}$$

Condensed computation of Example 2. (*Note:* The steps are performed in order according to the formulas.)

Solving for Z first:

```
            8.6       8.6
           -5.4       ×40          5.4
 40        ----      -----         ×4
 -4         3.2      344.0        ----
----       ×10½      -21.6..........21.6
 36        ----      -----
  10        320      322.4=bd-ae
----         16      ×.82
360        ----      -----
 33.6 .......33.6    25792
----                   645
393.6                -----
264.4...............264.37
----
129.2 Numerator of formula.
```

Finding the value of the denominator:

```
  36   Since any number times zero is
 ×32   zero the second term of the
-------denominator is Zero
  72   The third term is .58 times the
 108   bd-ae already found, i. e.,
-------.58×322.4=187.0.
1152
  1152-187.0=965, the Denominator.
```

Dividing the numerator by the denominator,

129.2÷965=.1339
1000× 1339=133.9 lbs. sweetened condensed skim.

Solving for Y:

.82
×4
3.28 .... 10 5
−3 28
7.22 × 1000 = 7220
.58
×4
2.32 ...... 133.9
..×2.32
40 310.6
−4 7220.0
36) 7539.6 (*209.2lbs. of cream.*

Check as before.

Solving for X:

10.5
×1000
10500.0
8368.0.................. 209.2
×40
.... .8368.0
4)2132.0
533 lbs. of milk. 133.9
×0
0000

Preliminary "check":

533.0 lbs. milk
209.2 lbs. cream
133.9 lbs. sweetened condensed skim
35.0 lbs. gelatin and water
88 8 lbs. sugar to be added (since the total amount of sugar is 145 lbs. and the 133.9 lbs. of condensed skim contains .42×133.9 or 56.2 lbs) 145−56 2=88.8.
999.9

The caution given in the foot note on page 63 about checking the results should be heeded here also.

The complete check follows:

As in the "check" for Example 1,
10.5% of 1000 lbs.=105 lbs. of fat required.
The sources of fat are the 4% milk and 40% cream.
4% of 533.0 lbs. = 21.32 lbs. fat from milk,
40% of 209.2 lbs. = 83.68 lbs. fat from cream.
105.00 lbs. Total fat, which checks.

10% of 1000=100 lbs. of M.S.N.F. required.

The sources of M.S.N.F. are 8.6% from milk, 5.4% from cream, and 32% from sweetened condensed skim.

8.6% of 533.0 lbs. = 45.84 lbs. M.S.N.F. in milk.
5.4% of 209.2 lbs. = 11.30 lbs. M.S.N.F. in cream.
32% of 133.9 lbs. = 42.75 lbs. M.S.N.F. in sweetened condensed.
99.89 lbs. Total M.S.N.F. which checks the required 100 lbs. nearly enough.

(It is probably superfluous to state that carrying the results out further would give a still closer check.)

Example 3. **(Plain Condensed Whole Milk)**

To make 1000 lbs. of standard mix with the following ingredients:

Milk, 3.4% fat
Cream, 38% fat
Plain condensed whole milk $\begin{cases}8\% \text{ fat}\\ 23\% \text{ M.S.N.F.}\end{cases}$

This example comes under Case II, but since the plain condensed whole milk contains no sugar, $k=100\%-0\%=1$, so that the last term in the denominator of the formula is just $1(bd-ae)=bd-ae$.

Henceforth only the short form of the solutions will be given.

Finding Z first:

$a=3.4$
$b=38$
$c=23$
From Table $\begin{cases} d=8.7 \text{ (since 3.4 milk is}\\ e=5.5 \text{ nearly 3.5)}\end{cases}$
$h=8$
$k=1$

$b-a=\begin{cases}38.0\\ 3.4\end{cases}$
34.6
×10
346
+33.6
379.6
−255.8
Num. = 123.8

8.7
5.5
3.2 $=d-e$
10½
320
16
33.6

Dividing Numerator by Denominator

123.8÷509.5=.2430
1000×.243=243 lbs. condensed milk=Z.

$b-a=34.6$
$c=\times 23$
1038
692
$c(b-a)=795.8$

$d-e=\ \ 3.2$
$h=\ \ \times 8$
$h(d-e)=\ \ 25.6$
$c(b-a)=795.8$ add
821.4
$k(bd-ae)=311.9$ since $k=1$
509.5 Denom.

$bd=\begin{cases}38\\ \times 8.7\\ 266\\ 304\\ 330.6\end{cases}$

$ae=\begin{cases}3.4\\ \times 5.5\\ 170\\ 170\\ 18.70\end{cases}$

330.6
−18.7
311.9 $=bd-ae$
×.82
24952
624
255.76

Finding Y:

$$
\begin{array}{rr}
 & a=3\ 4 \\
 & \times.82 \\ \cline{2-2}
 & 272 \\
 & 7 \\ \cline{2-2}
 & \left.\begin{array}{r}2.79\\ 10.50\end{array}\right\}\text{Subtract} \\ \cline{2-2}
 & 7.71 \\
 & \times1000 \\
akZ= & \left.\begin{array}{r}7710.00\\ 826.00\end{array}\right\}\text{Add} \\ \cline{2-2}
hZ= & \left.\begin{array}{r}8536.0\\ 1944.0\end{array}\right\}\text{Subtract} \\ \cline{2-2}
 & 6592.0=\text{Num.}
\end{array}
$$

$$
akZ=\left\{\begin{array}{r}243\\ \times3\ 4\\ \hline 972\\ 729\\ \hline 826.2\end{array}\right.
\qquad
hZ=\left\{\begin{array}{r}243\\ \times8\\ \hline 1944\end{array}\right.
$$

$b-a=34.6=$Denom.

6592÷34.6=190 52 lbs. cream

Finding X:

$$
\begin{array}{rr}
 & 10\ 5\times1000=10500 \\
 & 190\ 52 \\
 & \times38 \\ \cline{2-2}
 & 57156 \\
 & 15242 \\ \cline{2-2}
 & 7239.8 \\
 & \\
 & 10500.0 \\
 & 7239.8 \\ \cline{2-2}
 & 3260.2 \\
hZ= & 1944 \\ \cline{2-2}
 & \mathit{3.4})\ 1316.2\,(\mathit{387.1}\ \text{lbs. milk.}
\end{array}
$$

Preliminary check:

.82×1000=820 lbs. dairy products

$$
\begin{array}{r}
243\ 0\\
190.5\\
387.1\\ \hline
820.6
\end{array}
$$

which checks nearly enough.

Complete check:

3.4% of 387.1 lbs.=13.16 lbs. fat from milk.
38% of 190 5 lbs.=72.40 lbs. fat from cream.
8% of 243 0 lbs.=19 44 lbs fat from condensed milk
105.00 check.

10% of 1000 lbs.=100 lbs. M.S.N.F. required.

Three sources, 8.7% in milk, 5.5% in cream, and 23% in condensed whole milk.

8.7% of 387.1 lbs.= 33.67 lbs. from milk,
5 5% of 190.5 lbs.= 10.48 lbs. from cream,
23% of 243.0 lbs.= 55 89 lbs. from condensed whole.
100.04 check.

The most general problem under Case II, arises when sweetened condensed whole milk is used. Then both h and k have special values in the formulas.

Example 4. **(Sweetened Condensed Whole Milk)**

To make 1000 lbs. of standard mix using:

$$
\text{Sweetened condensed whole}\left\{\begin{array}{l}8\%\ \text{fat}\\ 23\%\ \text{M.S.N.F.}\\ 40\%\ \text{sugar}\end{array}\right.
$$

Milk 4%
Cream 35%

$a=4$ $h=8\ 0$
$b=35$ $k=100\%-40\%=.60$
$c=23$

From Table $\left\{\begin{array}{l}d=8.8\\ e=5.8\end{array}\right.$

Substituting in the general formulas of Case II, page 66:

$$Z=\frac{10(35-4)+10\ 5(8\ 8-5\ 8)-\ 82(35\times 88-4\times 5.8)}{23(35-4)+8.0(8.8-5.8)-.60(35\times 8.8-4\times 5.8)}\times 1000.$$

Note: The numerator for Z is the same as in Example 1, page 63, and, hence, equals 108.0.

For the denominator:

```
b−a= { 35
     { −4
     ────
       31           d−e= { 8.8        3.5
       23                { 5.8        8 8
     ────                ─────       ─────
       93                  3 0        280
       62                 ×8 0        280          5.8
     ────                ─────       ─────          ×4
      713 .  .....   .    24.0       308.0         ────
                          713 0       23.2 ...    .23 2
                         ─────       ─────
                          737 0      284.8
                                       .60
                                     ─────
                          179.9 ......170.88
                         ─────
                          566.1=Denom.
```

108.0÷566.1=.1908

1000×.1908=190 8 lbs. sweetened condensed whole.

$$Y=\frac{(10.5-\ 82\times 4)\times 1000+4\times .60\times 190\ 8-8\times 190.8}{35-4}.$$

```
 .82
 ×4                               190.8
────                               ×.60
 3.28                             ──────
10.5                              114.480
────                                 ×4
 7.22×1000=7220.0                 ──────
            +457.9 ...... ...... .. 457.92              190.8
            ──────                                        ×8
             7677.9                                     ──────
            −1526 4. ..... ........ .... .. ... ... .. ..1526.4
            ──────
```

31)6151.5(*198 4 lbs. cream.*

$$X=\frac{10\ 5\times 1000-35\times 198.4-8\times 190.8}{4}.$$

```
                 189 4
                  ×35
10.5             ─────
 ×1000            5952
──────             992
10500            ─────
 6944 . .. ....... 6944          190 8
──────                              ×8
 3556                             ─────
 1526.4 ........ ........ .........1526.4
──────
4)2029.6
 ──────
  507.4 lbs. milk
```

Check: Here the sweet condensed whole includes sugar equal to 40% of 190.8 or 76.32. Hence, only 190.8−76.3 or 114.5 lbs. of it is strictly "dairy products".

114.5+198.4+507.4=820.3 lbs. which checks the 820 lbs. dairy products required.

4% of 507.4=	20.30	lbs. from milk,
8% of 190 8=	15.25	lbs. from sweet condensed whole,
35% of 198.4=	69 44	lbs. cream.
	105.00	which checks fat needed.
8 8% of 507.4=	44 65	lbs. from milk,
23% of 190.8=	43.88	lbs. from condensed whole,
5.8% of 198 4=	11 51	lbs. from cream.
	100.04	lbs. which checks M.S.N.F. needed.

Miscellaneous.

Under this head are classed those problems in which some of the ordinary ingredients are missing or given amounts of some of them are used.

Example 5. **(Cream and Condensed Skim only)**

To make 1000 lbs. of mix using only cream and condensed skim (no whole milk) so that $a=0$ and $d=0$.

$a=0$
$b=40$
$c=30$
$d=0$
$e=5.35$

This problem comes under Case I, since h and k do not appear; and the formulas for Z and Y become:

$$Z=\frac{10b-10.5e}{cb}\times M;\qquad Y=\frac{10.5M}{b}.$$

Finding Z:

	6.2	40
40	×10½	30
×10	620	1200 Denom.
	31	
400		
65.1	65.1	

334.9÷1200=.2791
1000×.2791=279.1 lbs. condensed skim

Finding Y:

10.5×1000=10500
10500÷40 =262 5 lbs. cream,
279.1 lbs. skim.

541.6 lbs. dairy products.

But since only 18% of the mix is accounted for in the sugar, gelatin and water, we must still account for 82% of it or 820 lbs. Hence, we must add water enough to make this amount.

820
541 6

278.4 lbs. water to be added.

Example 5a. **(Maximum Amount of Cream)**

The maximum amount of cream will be used if cream only is used as a source of fat and this problem is solved in example 5 above. Of course, if the cream is very rich, say 30 to 40%, water will have to be added. Example 5 shows how to compute the amount of cream and condensed skim and how to find the amount of water to be added.

Example 6. **(Condensing Problem)**

No condensed product is used, but the mix is first made up larger than it should be finally and then condensed. Since no condensed product enters into this mix, there is no formula for Z. The formulas for X and Y follow:

$$X=\frac{10b-10.5e}{bd-ae}\times M \qquad Y=\frac{10.5d-10a}{bd-ae}\times M$$

where the letters have the usual meanings, as explained on page 61.

To make 1000 lbs. of standard mix using 4% milk and 40% cream and condensing down, thus:

$a=4$ $\quad d=8.8$
$b=40$ $\quad e=5.35$ } From table
$c=0$ (does not appear)

Substituting in the formulas for X and Y:

$$X=\frac{10\times40-10.5\times5.35}{40\times8.8-4\times5.35}\times1000=\frac{400-56.17}{352-21.4}\times1000$$

$$=\frac{343.83}{330.6}\times1000=1040 \text{ lbs. milk}$$

$$Y=\frac{10.5\times8.8-10\times4}{330.6}\times1000=\frac{92.4-40}{330.6}\times1000$$

$$=\frac{52.4}{330.6}\times1000=158.5 \text{ lbs. cream.}$$

1040 lbs. milk
158.5 lbs. cream
180 lbs. sugar, gelatin and water (=18% of 1000 lbs.)

1378.5 Total to be condensed down to 1000 lbs.

Example 7. **(Cream, Skim and Condensed Skim)**

To make 1000 lbs. standard mix with following ingredients.

30% cream,
Plain skim, 8.9% M.S.N.F.
32% condensed skim

The formulas of Case I, page 62, are used, where the skim milk takes the place of the whole milk.

Hence,

$a=0$
$b=30$
$c=32$
$\left.\begin{array}{l} d=8.9 \\ e=6.2 \end{array}\right\}$ $2\ 7=d-e.$

The formulas become:

$$Z=\frac{10b+10.5(d-e)-.82\ bd}{b(c-d)}\times M.$$

$$Y=\frac{10.5M}{b}. \qquad X=.82M-(Y+Z).$$

Substituting in the formulas,

$$Z=\frac{10\times30+10.5\times2.7-.82\times30\times89}{30(32-8.9)}\times1000.$$

	10.5	.82	32.0
	2.7	×30	8.9
30			
10	735	24.60	23.1
	210	8.9	×30
300			
28.35	28.35	2214	Denom. *693.0*)109.41(*.1579*
		1968	
328.35			
218.94		218.94	1000×.1579=157.9 or 158 lbs. condensed skim.
Num. 109.41			

Finding Y:

$$Y=\frac{10.5\times1000}{30}=\frac{10500}{30}=350 \text{ lbs. cream.}$$

$$\begin{array}{r} 350 \\ +158 \\ \hline 508 \text{ lbs.} \end{array}$$

$.82\times1000=820$
$X=820-508=312$ lbs. skim.

Check: 30% of 350 lbs. = 105 lbs. fat from cream, which checks, as the cream is the only source of fat.

M.S.N.F. { 6.2% of 350 lbs. = 21.70 from cream.
8.9% of 312 lbs. = 27.77 from skim,
32% of 158 lbs. = 50.56 from condensed skim.

100.03 lbs. total which checks 100 lbs. required.

Example 8. (**Left Overs:** One may have a certain amount of cream or milk or both left over which must be used in the mix.)

To make 1000 lbs. standard mix using:

60 lbs. left over 35% cream,
50 lbs. left over 20% cream,
150 lbs. left over 4% milk,
and enough 3.8% milk and 40% cream and 30% condensed skim to make the mix. It is necessary to find first how much fat and M.S.N.F. are contained in the milk and cream left over which must go into the mix.

35% of 60 lbs. = 21.0 lbs. of fat from cream,
20% of 50 lbs. = 10.0 lbs. of fat from cream,
3.8% of 150 lbs. = 5 7 lbs. of fat from milk,
36.7 lbs. fat accounted for.

10.5% of 1000 = 105.0 lbs. fat required altogether.
105.0 lbs. − 36.7 = 68.3 lbs. still needed.
68.3 ÷ 1000 = .0683 or 6 83%; that is, the fat still needed equals 6.83% of the 1000 lbs. mix.
Hence, the 6.8% (close enough) takes the place of the old 10.5 in the formulas of Case I.

Similarly for M.S.N.F.

5.8% of 60 lbs. = 3.48 M.S.N.F. from cream.
7.1% of 50 lbs. = 3.55 M.S.N.F. from cream.
8.8% of 150 lbs. = 13.20 M.S.N.F. from milk.
20.23 = M.S.N.F. accounted for.

10% of 1000 lbs. = 100 lbs. M.S.N.F. required altogether.
100 − 20.23 = 79.77 or 80 lbs. M.S.N.F. still needed.
80 ÷ 1000 = .080 or 8%.

Hence the 8.0 takes the place of the old 10 in the formulas.

Now, as usual with the standard mix, 82% of it consists of dairy products amounting to 820 lbs. The 60+50+150 to be used amounts to 260 lbs.

Hence, 820 lbs. − 260 lbs. or 560 lbs. equals amount still needed. 560 ÷ 1000 = .56 which takes the place of the old .82 in the formulas.

Hence, the formula for Z, Case I, page 62, is:

$$Z = \frac{8.0(b-a)+6.3(d-e)-.56(bd-ae)}{c(b-a)-(bd-ae)} \times M$$

$$Y = \frac{(6.8-.56a)M+aZ}{b-a}$$

$$X = \frac{6.8M-bY}{a}$$

The problem now is to find how much of the 3.8% milk, 40% cream and 30% condensed skim must be used to make up the 560 lbs. still needed, which is done by substituting the proper values of a, b, etc., in the formulas as written above.

In this particular example:

$a=3.8$
$b=40$
$c=30$
From Table $\begin{cases} d=8.8 \\ e=5.35 \end{cases}$

40 0
3.8
36.2
×8
289 6
+23.5
313.1
185 75
127.35=Num.

$d-e=\begin{cases} 8.80 \\ 5.35 \\ 3.45 \end{cases}$
×6.8
2070
276
23.45

$bd=\begin{cases} 8\ 8 \\ \times 40 \end{cases}$
352.0

$ae=\begin{cases} 5\ 35 \\ \times 3.8 \end{cases}$
1605
428
20.33
352.0
−20.3
331.7 $=bd-ae$
×.56
16585
1990
185.75

Finding Denom.:

$c(b-a)=\begin{cases} 36.2 \\ \times 30 \end{cases}$
1086.0
$bd-ae$ 331.7
754.3=Denom.
127.35÷754.3=168 8 lbs. condensed skim required.

Finding Y:

.56
3.8
168
45
2.13
6.80
2.13
4.67×1000=4670.0

168.8
3.8
5064
1350
641.4
641.4

$b-a=$*36.2*)5311.4(*146.7* lbs. cream.

Finding X:

6.8×1000=6800.0
bY=40×146.7=5868.0
3.8)932.0(*245.3* lbs. milk

Check: 245.3
146.7
168.8
560.8 lbs. which checks the 560 lbs. needed (near enough.)

Fat $\begin{cases} \text{40\% of 146 7 } = 58.68 \text{ from cream,} \\ \text{3.8\% of 245.3} = \ \ 9.32 \text{ from milk,} \end{cases}$
68.00 Total,
which checks the 68.3 lbs. needed:

30% of 168.8=50.65 lbs. from skim,
5.35% of 146.7= 7.85 lbs. from cream,
8.8% of 245.3=21.60 lbs. from milk,
80.09 lbs. Total M.S.N.F.,
which checks the 80 lbs. needed.

Example 9. **(Having to use a given amount of Sweet Butter and Cream)**

To make 1000 lbs. of standard mix, using
100 lbs. sweet butter, 83% fat;
50 lbs. of 25% cream with enough 40% cream (no whole milk) and 32% condensed skim to make the mix.

It is necessary first to find how much fat and M.S.N.F. the given ingredients contain, then to find how much must be furnished by the unknown 40% cream and condensed skim.

83% of 100 lbs. = 83 lbs. fat in the butter.
25% of 50 lbs. = 12.5 lbs. fat in the 25% cream.
95.5 lbs. fat in both.

10.5% of 1000 lbs. = 105 lbs. in the mix.
105 − 95.5 = 9.5 lbs. fat still needed.

Since this 9.5 lbs. of fat is to come from 40% cream, 9.5 ÷ .40 = 23.75 lbs. of 40% cream needed.

100 + 50 + 23.75 = 173.8 lbs. total cream and butter.

Now, in order to use the formulas, and thus avoid "cutting and trying," it is necessary to find what this cream and butter test, both in fat and M.S.N.F.

105 ÷ 173.8 = 60.4% fat = b in the formulas.

The 25% cream tests 6.68% or 6.7% M.S.N.F. by the table and the 40% cream tests 5.35%

6.7% of 50 lbs. = 3.35 lbs. M.S.N.F. in 25% cream.
5.35% of 23.75 = 1.27 lbs. M.S.N.F. in 40% cream.
Total = 4.62 lbs. M.S.N.F. in cream and butter.

Now since no milk is used, a and e are Zero (= 0).

Hence:

$a=0$ $d=0$
$b=60.4$ $e=2.65$
$c=32$

Y, the cream and butter is already known, namely 173.8 lbs. and the formula for Z becomes:

$$Z=\frac{10b-10.5e}{cb}\times M.$$

Substituting the new values of b and e in the formulas for Z,

$$Z=\frac{10\times60.4-10.5\times2.65}{32\times60.4}\times1000.$$

	26.5	
	×10½	
60.4		60.4
×10	26.5	32
	1.32	
604.0		1208
27.82	27.82	1812
Num. = 576.18		1932.8 = Denom.

576.18 ÷ 1932.8 = .298
1000 × .298 = 298 lbs. condensed skim.
Check as usual.

Special Problem in which given amounts of two or three ingredients are used, and which can be solved without either the algebraic formulas or "cutting and trying."

Example 10. **(Given amounts of two or more ingredients)**

To make 1000 lbs. of standard mix with the following given amounts of milk and cream.

200 lbs. of 4% milk
100 lbs. of 3% milk
100 lbs. of 35% cream and enough 40% cream and 30% skim to make the mix.

Solution:

4% of 200 lbs. = 8 lbs. fat,
3% of 100 lbs. = 3 lbs. fat,
35% of 100 lbs. = 35 lbs. fat.
46 lbs. fat in given ingredients.

105 lbs. − 46 lbs. = 69 lbs. fat still to come from 40% cream.
69 ÷ .40 = 172.5 lbs. of 40% cream needed.

8.8% of 200 lbs. = 17.6 lbs. from 4% milk,
8.6% of 100 lbs. = 8.6 lbs. from 3% milk,
5.8% of 100 lbs. = 5.8 lbs. from 35% cream,
5.35% of 172.5 = 9.2 lbs. from 40% cream.
41.2 lbs. provided for.

100 lbs. − 41.2 = 58.8 lbs. M.S.N.F. still to come from condensed skim testing 30%. 58.8 lbs. ÷ .30 = 196 lbs. condensed skim.

Hence 172.5 lbs. of 40% cream and 196 lbs. of condensed skim are needed in addition to the given ingredients.

Other Standards: The formulas and rules given above can be applied to other standard mixes than the one used thus far in this bulletin.

Example: To make 1000 lbs. (or any other amount) of mix which shall contain the following:

12% fat.
11% M.S.N.F.
14% sugar.
0.5% gelatin with no water.

14% sugar+0.5 gelatin=14.5%.

Hence, the dairy products must constitute 100%−14.5%=85.5% or .855 of the mix and the numbers 12, 11, and .855 simply take the place of 10.5, 10 and .82 in the formulas of Case I and Case II. Thus the formulas of Case I become:

$$Z=\frac{11(b-a)+12(d-e)-.855(bd-ae)}{c(b-a)-(bd-ae)}\times M,$$

where a, b, c, etc., have the same meaning as in previous examples, and M is 1000 or 750 or any other amount of mix desired.

$$Y=\frac{(12-.85a)M+aZ.}{b-a}$$

$$X=\frac{(12M-bY).}{a}$$

The rules on pages 64 to 66 apply here also.

Thus in step 2, the result of step 1 is multiplied by 11 under this standard, instead of 10: and in step 9, the result of step 8 is multiplied by .855 instead of .82, and so on.

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THE RELATION OF STORED FOOD TO CAMBIAL ACTIVITY IN THE APPLE

E. L. PROEBSTING

INTRODUCTION

This paper reports an attempt to determine, by defoliation methods, to what extent cambial activity and storage of food are correlated. There have been observations along this line for many years, which have been ably summarized by Knudson,[7] Harper,[9] Grossenbacher,[3] André,[1] and Robbins.[11] The present state of knowledge, as judged from these and other less intimately related papers, seems to be a well-defined idea that cambial activity is dependent on leaf activity.

Wieler[14] found that covering three or four-year-old trees of *Quercus sessiliflora* before leaf development in spring, by means of boxes lined with black paper, caused a reduction in diameter growth as compared with checks. Jost (cited by Grossenbacher[3]), removed the terminal buds and thereby caused failure of radial growth in pine. Harper[4] observed the effects of successive defoliations of the larch by insects which caused first, a reduction in thickness of the cell walls and then, a decrease in the width of the annual rings until, in a few years, growth ceased entirely.

A report by Harvey[5] which is closely allied, and in some respects very similar, to the paper here presented, has recently appeared. Defoliation was found to accelerate or retard growth as measured by terminal elongation, according to the stage of development of the shoot. This criterion of growth makes direct comparison difficult

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[*] Also presented to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

between Harvey's work and that here reported. There may not be a particularly good correlation between terminal and lateral growth. (See tables 8 and 9 below.) Differences in methods and materials also may account for the somewhat different results obtained. However, the hypothesis he developed for the unification of his results, that is, the carbohydrate-nitrogen ratio, should be generally applicable. This phase will receive attention later in this paper under Discussion and Conclusions.

The above citations include the chief contributions having an immediate bearing on the present problem. With the exception of the work of Harvey, the results are practically all "qualitative" in character. Quantitative data on the checking of diameter increase by defoliation were considered necessary as a foundation for any further development of the facts involved in this phenomenon, particularly with regard to change in composition that might prove to be associated with it.

MATERIALS AND METHODS

For the purpose of this investigation, trees were selected in the spring of 1922 from a six-year-old McIntosh orchard at the New York Agricultural Experiment Station, at Cornell University, Ithaca, New York. Because of the youth of the trees, there was no fruit production to interfere with vegetative relationships. Four of the trees had all of their leaves removed as soon as they appeared in the spring, that is on May 3. As new leaves appeared subsequently, they were also removed. Another group of four trees were allowed to grow normally until June 6, when they also were defoliated and kept bare as in the previous series. Four other trees were half defoliated, i.e., had all the leaves from half of the tree removed, on the same dates as the whole trees. Material was collected from these trees at intervals of from one to two weeks from the latter part of April until September 8, 1922, and consisted usually of ten one-year-old twigs from each tree.

Portions of each twig were taken from the apical, middle and basal regions, each about 1 cm. in length. The remainder of each twig was prepared for analysis as described below. These 1 cm. pieces were killed in 50 per cent alcohol. The alcohol was removed by washing in water, and the pieces were then treated with hydrofluoric acid for two weeks. The acid was then washed out with water, and the pieces placed in glycerin and alcohol (one-half glycerin, one-half 95 per cent alcohol, by volume). They were sectioned from this mixture, without imbedding. It was found that sections twenty-five to thirty-five microns in thickness could be readily cut in this way, and that this was thin enough for the purposes of this work. This method did not permit, however, any measurements being made on the bark other than its total thickness. Safranin and haematoxylin were used for staining in most cases, phloroglucin being used for some of the temporary mounts.

MEASUREMENT OF CAMBIAL ACTIVITY

Each section was measured by means of an ocular micrometer. Data were recorded for the thickness, measured along a radius, of the pith, one-year-old wood, new wood and bark. About fifteen sections were placed on each slide and were measured for each of the three pieces from each twig. An average for each slide was arrived at which was a mean of fifty to seventy-five measurements. The means of new wood measurements were then grouped and treated statistically, their mean with its probable error being ascertained (table 1). The differences between those of the check and those of the other series collected on the same day are given in table 2. The means of these classes are plotted, for the new wood, in figure 1. The data for the halves of trees are given in table 3.

TABLE 1

EFFECT OF DEFOLIATION ON RADIAL GROWTH IN NEW WOOD OF ONE-YEAR-OLD TWIGS OF McINTOSH APPLE IN 1922

Date	Check	Defoliated May 3	Defoliated June 6
	Microns	*Microns*	*Microns*
Apr. 19	....		
May 3	13.9 ± 2.5		
May 12	72.7 ± 3.9	66.0 ± 2.8	
May 24	266.3 ± 9.2	134.6 ± 6.3	
June 6	480.2 ± 15.7	116.6 ± 5.1	
June 20	582 5 ± 17.9	113.4 ± 5.5	475.6 ± 11.7
June 30	543.5 ± 14.4	140.1 ± 7.4	398.2 ± 11.7
July 11	927.0 ± 34.1	123.1 ± 2.1	492.5 ± 14.9
July 20	913.7 ± 29.9	130.0 ± 7.0	567.2 ± 12.8
Aug. 4	1100.6 ± 31.9		545.1 ± 12.8
Aug. 18	781.8 ± 25.6		
Sept. 8	819.9 ± 37.3	133.5 ± 5.2	519.7 ± 14.9

These data are shown graphically in figure 1.

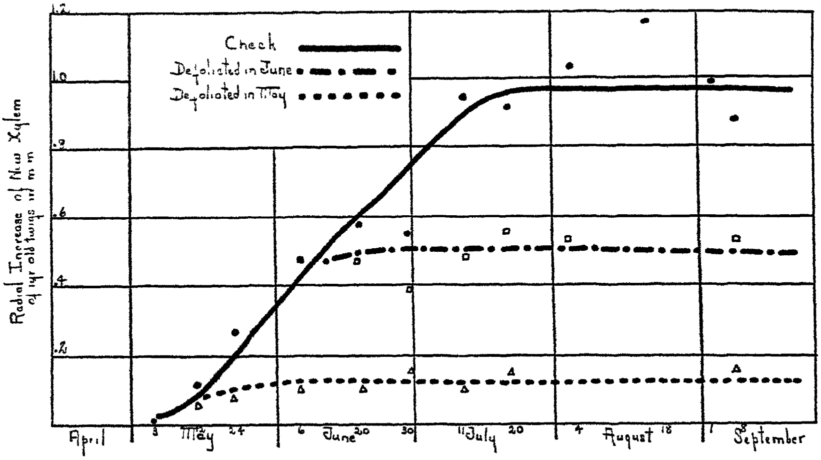

Fig. 1. The effect of defoliation on radial increase of new wood of one-year-old twigs of McIntosh apple.

TABLE 2

Differences Between Treated and Check Series, and Between Treated Series

Date	Difference between check and defoliated May 3	Difference between check and defoliated June 6	Difference between defoliated May 3 and defoliated June 6
	Microns	*Microns*	*Microns*
May 12	6.7 ± 4.8		
May 24	131.7 ± 11.4		
June 6	363.6 ± 16.5		
June 20	469.1 ± 18.7	106.9 ± 18.7	362.2 ± 12.9
June 30	403.4 ± 16.2	145.3 ± 18.5	258.1 ± 13.4
July 11	803.9 ± 34.2	434.5 ± 37.2	369.4 ± 15.0
July 20	783.7 ± 30.7	346.5 ± 32.5	437.2 ± 14.6
Aug. 4		555.5 ± 34.3	
Sept. 8	686.4 ± 37.6	300.2 ± 40.1	386.2 ± 15.8

TABLE 3

Measurements on Check and Defoliated Halves of Trees of McIntosh Apple in 1922—Measurements of New Growth of One-Year-Old Twigs

Date	Check half	Defoliated half	Difference
	Microns	*Microns*	*Microns*
June 8*	460.4 ± 17.9	135.4 ± 9.6	325.0 ± 20.3
June 26†	630.9 ± 22.4	521.8 ± 33.5	109.1 ± 40.3
June 28*	944.8 ± 31.9	152.8 ± 12.8	792.0 ± 34.4
July 31*	1274.6 ± 68.3	148.5 ± 19.2	1126.1 ± 70.9
Aug. 10†	1237.6 ± 45.8	635.3 ± 25.6	602.3 ± 52.4

* Defoliated May 3. † Defoliated June 6.

Caliper measurements were made on two defoliated and two check trees. The data (table 4) show the same tendencies as those obtained by micrometer measurements up to the middle of July. After that there was an increase in the check, due probably to maturation and swelling of bark tissue.

TABLE 4

DIAMETER OF TWIGS AS SHOWN BY CALIPER MEASUREMENTS IN MILLIMETERS

Date	Check trees		Defoliated trees	
	2A	1C	3A	4A
May 3	4.83 ± .25	6 28 ± .47	5.46 ± .41	5.80 ± .21
June 6	5.73 ± .33	7.02 ± .49	5.66 ± .42	5.92 ± .23
June 26	6.20 ± .29	7 24 ± .52	5.68 ± .42	5.98 ± .23
July 20	6.30 ± .29	7.70 ± .59	5 66 ± .42	5 98 ± .23
Aug. 18	6.51 ± .33	8 02 ± .65	5 58 ± .40	5.96 ± .23
Sept. 8	6 80 ± .33	8.32 ± .59	5 64 ± .42	5.96 ± .23

The results recorded in tables 1, 2, 3, and 4 show clearly that growth is entirely checked within about two weeks as a result of defoliation. It is obvious that the growth that did occur was due to food from the stored reserve, since none was available from new leaves. Furthermore, the cessation of growth was not due to the death of the twigs, since they were able to continue to push out new leaves until the last of summer.

In order to be certain that the error due to unconscious selection of larger twigs in one series than in another was not a factor, the radii, exclusive of new wood, were compared. It was found that the average difference was 5±53 microns. This may be taken to show conclusively that differences in twig size are due to random sampling only, and play no significant part in the results reported.

A second set of trees in the same orchard was defoliated on May 4, 1923, as soon as the leaves unfolded. The methods were the same as those employed the year before. Collections were made on May 4 and 28 and on June 12. The data for this material are given in table 5.

TABLE 5

EFFECT OF DEFOLIATION ON THE RADIAL GROWTH OF NEW WOOD OF THE ONE-YEAR-OLD TWIGS OF MCINTOSH, 1923

Date	Check	Defoliated	Difference
	Microns	*Microns*	*Microns*
May 4	0.0		
May 28	154.0 ± 5.80	96.0 ± 5.10	58.0 ± 7.70
June 12	347.0 ± 13.60	138.0 ± 6.10	209.0 ± 14.90

Another set of data was taken on different material. In this case one-year-old apple seedlings were grown in the greenhouse. This experiment was not highly successful because of the low percentage of trees that was brought out of the rest period at nearly enough the same date to be comparable. The trees were handled as follows:

November 11, 1922—Dug and heeled in out-of-doors.
January 30, 1923—Heeled in under greenhouse bench.
February 27, 1923—Potted in garden soil, ten trees to a seven-inch pot.

TABLE 6

RADIAL GROWTH OF NEW WOOD OF ONE-YEAR-OLD SEEDLINGS GROWN IN GREENHOUSE, 1923

Date	1 Check	2 Defoliated throughout	3 Defoliated from April 13 on	4 Defoliated to April 13 then allowed to grow	5 Same as Column 4 except defoliated again after May 15	6 Same as Column 3 except allowed to grow again after May 15	7 Buds removed as growth started
Apr. 25	134±18.3						
May 7	158±13.4	83±15.6					15±3.2
May 25	219±20 6				144±13.4	160±21.6	
May 30		115±12 4					63±7.8
June 7	198±21.2	111±10.0	111±16.3	266±21.1	134±11.5	169±19.3	

Of a total of nearly five hundred trees potted, about a third had to be discarded completely. Those remaining were divided into several series, as follows:

1. Check; no treatment.
2. Defoliated as in the experiments recorded above.
3. Defoliated after having grown normally until April 13.
4. Defoliated until April 13, and then allowed to grow normally.
5. The same as 4 until May 15, then defoliated again.
6. The same as 3 until May 15, then allowed to grow normally again.
7. All of the buds removed before they had unfolded.

Collections were made at irregular intervals and treated as in the earlier experiment. There was a much higher variability in this material than in the material taken from the orchard. This fact may perhaps be ascribed to genetic differences among the seedlings, as contrasted with the homogeneous constitution of the other population. This high variability rendered differences less obvious. Such differences as were found were, with a single exception, in the direction of the results recorded above. The data obtained from these seedlings are of little value by themselves, but tend to support the other data. The data from the sections of these trees are found in tables 6 and 7. The most striking result appears in series 7, that having the buds removed, where a large number of trees failed to survive the treatment. Of those that did survive few made more than a slight amount of growth.

TABLE 7

DIFFERENCES BETWEEN CHECK AND TREATED TREES

(Treatments numbered as in table 6)

Date	Treatment	Difference from check in microns
May 7	2	75 ± 20.5
May 7	7	143 ± 13.8
May 25	6	59 ± 29.8
May 25	5	75 ± 24.5
June 7	2	87 ± 23.4
June 7	3	87 ± 26.7
June 7	6	29 ± 28.6
June 7	4	+68 ± 29.9
June 7	5	64 ± 24.1

TABLE 8

TERMINAL GROWTH OF SEEDLINGS

Date	Series	Number of trees	Number of buds	Total growth (Cm.)	Growth per tree (Cm.)	Growth per bud (Cm.)
Mar. 28	1	49	247	271	5.5	1.09
Mar. 28	2	10	34	23	2.3	.67
Mar. 28	3	4	17	17	4.3	1.00
Mar. 28	4	12	71	37	3.1	.52
Mar. 28	6	17	77	71	4.2	.91
Mar. 28	5	20	95	72	3.6	.76
Apr. 13	1	61	292	601	9.8	2.13
Apr. 13	2	27	139	150	5.5	1.08
Apr. 13	3	5	24	14	3.8	.58
Apr. 13	4	16	104	308	19.2	2.96
Apr. 13	6	20	94	107	5.3	1.14
Apr. 13	5	29	152	385	13.3	2.53
May 7	1	15	99	303	20.2	3.06
May 7	2	8	55	130	16.2	2.36
May 15	1	37	144	560	15.1	3.88
May 15	2	32	137	309	9.7	2.25
May 25	6	10	71	268	26.8	3.77
May 25	5	10	92	240	24.0	2.61

In addition to those of radial increase, measurements were made of terminal growth on this material. Some of these measurements are given in tables 8 and 9.

TABLE 9

COMPARISON OF RADIAL AND TERMINAL GROWTH

Date	Series	Tree No.	Total terminal growth per tree (Cm.)	Number of buds	Growth per bud	Radial growth (Microns)
May 7...	Check	I	10	4	2.5	166.0
		II	7	8	.88	141.1
		III	35	8	4.38	224.1
		IV	25	7	3.77	107.9
		V	15	1	15.00	141 1
		VI	30	12	2.50	74.6
		VII	30	15	2.00	149.4
		VIII	30	4	7.50	249.0
		IX	10	4	2.50	166.0
		X	20	6	3 33	166.0
		Average	21.2±2.16		4.44± 877	158.5±33.7
May 7....	Defoliated	I	10	5	2 00	49.0
		II	12	2	6.00	33.2
		III	...	..	....	.....
		IV	18	6	3.00	66.4
		V	20	12	1.67	99.6
		VI	28	18	1.55	24.9
		VII	25	5	5.00	58.1
		VIII	2	5	.40	24.9
		Average ...	16.4±2.29		2.80±.513	50.9± 6.8
		Difference..	4.8±3.14		1.64±1.016	107.6±35.8

No definite correlation can be seen between radial and terminal growth, either total or per bud, nor was terminal growth governed so closely by the treatment. This lack of correlation may be due to the high variability of the material, however, and cannot be given too much weight. The fact that Harvey found a correlation between length growth and treatment tends to discount these results, since he worked within a clone, and should have had much more uniform material. The fact that terminal growth can continue after lateral growth has ceased indicates either a difference in ability to utilize such food as is present or a distribution of food in greater concentration to the terminal meristems.

The appearance of the wood formed after defoliation was much like that observed in the larch by Harper, viz., that it was thin-walled and resembled spring wood. This was especially noticeable in the series defoliated June 6, 1922. This type of wood was formed after summer wood formation had begun. There was no evidence that walls, thickened before defoliation, lost any material afterwards. Spring wood was also quite noticeable in series 6, which had two growth periods, where false annual rings were common.

A final set of material was collected from Red Astrachan apple trees located at the University Farm, Davis, California, in the spring of 1924. The period covered by this series was from March 7 to April 22, the critical period in the differentiation of the treated and untreated series. These trees were five years old. All treatments were given to halves of trees. Sufficient sections were made to determine that the response was the same as in the previous material sectioned.

These facts bring out clearly that cambial activity is dependent on the presence of leaves for its normal functioning. There is lacking the ability to function without the coöperation of the leaves.

The remainder of the work presented deals with the analysis of the material collected to determine whether or not any one of the groups: reducing sugars, non-reducing sugars, starch, hemi-celluloses or total nitrogen, can be designated as the limiting factor of growth under these conditions.

METHODS OF CHEMICAL ANALYSIS

Preparation of Material

The collections from the orchard made in 1922 were separated into wood and bark before further treatment was given. After finding that the curves for the two sets of analyses largely paralleled each other, it was decided to analyze the later series without this separation. This proved to be unwise, since the varying proportions of wood and bark introduced a very large error and the variability was found to be excessive. The material collected in California was divided into wood and bark. It is apparent, therefore, that the greater weight must be given the 1922 series and the 1924 series.

The wood and bark, and later the twigs as a whole, were dried at 78° to 80° C. This material was then ground to a coarse dust. A simple and very satisfactory device was used for grinding the wood. A large pencil sharpener was connected to a motor through a reduction gear. This gave a fairly uniform coarse powder that was fine enough to be readily extracted and coarse enough to be held by the alundum extraction thimbles. The bark was ground in a small coffee mill.

ANALYTICAL PROCEDURE

For sugars, it was found that three hours' extraction in a Soxhlet extraction tube, with 95 per cent alcohol, gave complete removal. The time was determined by repeating the extraction with a second portion of alcohol, and determining the time beyond which no further power of reducing Fehling's solution was evident in the second extract. After extraction, the usual process of replacing the alcohol by distilled water, precipitation with neutral lead acetate, deleading with sodium sulphate, carbonate or oxalate, and estimation of reducing sugars by means of the Munson and Walker gravimetric method, was followed. It was found that filtering the solution through asbestos in a Gooch crucible gave such satisfactory elimination of organic materials that oxidation of the cuprous to cupric oxide gave no better results.

Total sugars were estimated by the same method, except that inversion of disaccharides was necessary. For this process the usual procedure of heating with normal hydrochloric acid to seventy degrees for fifteen minutes, followed by neutralization with sodium carbonate, was followed.

Each analysis is a composite of all twigs of each series collected on a given date. The average of duplicate determinations is given in each case except for those indicated, where a single sample was available. The variability in sampling was apparently insignificant. Starch extraction was found to be satisfactorily accomplished by a slight modification of the method of Sablon.[13] The material, after the sugars had been extracted, was freed from alcohol. The residue was wet with distilled water and autoclaved for two hours at fifteen pounds pressure. The time, two hours, was arrived at by experiment. Different lengths of time were tried, the other factors being the same. After extracting the products of digestion, the process was repeated. It was found that an hour and a half was about on the borderline between giving nothing and giving a slight amount above the blank. It seems necessary to partially disrupt the cells in order to allow the enzyme to come into contact with the starch. The material was cooled. Taka-diastase was added and the mixture allowed to stand overnight at room temperature. One hundred milligrams of taka-diastase were used in each case. At this point every sample was examined microchemically to see whether or not the starch had all disappeared. In a very few cases it was necessary to repeat the process before going farther.

The solution was separated from the residue and treated as for total sugars. The acid hydrolysis was deemed necessary because Davis and Daish[2] found dextrins to be present after digestion and to be removed to a certain extent by the process of precipitation.

Hemi-celluloses were estimated by digesting the residue from the starch extraction in normal hydrochloric acid under a reflux condenser for three hours. The acid was neutralized with sodium carbonate and the solution treated as for reducing sugars.

Total nitrogen was determined by means of the phenol-sulphuric modification of the Kjeldahl method.

RESULTS OF CHEMICAL ANALYSES

1. *Reducing Sugars.*

The results from the determination of reducing sugars, expressed as dextrose in per cent of dry weight, are given in table 10.

TABLE 10

EFFECT OF DEFOLIATION ON THE REDUCING SUGAR CONTENT OF ONE-YEAR-OLD TWIGS OF MCINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Percent of dry weight as dextrose					
	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
June 6	.49	1.66	.18	.84		
June 20	.60	2.61	.17	1.14	.47	1.65
July 11	.47*	2.16*	.22*	1.16*	.25*	1.04*
July 20	.41*	1.61*	.27*	1.65*	.45*	1.66*
Aug. 4	.26	1.66			.29	1.48
Aug. 18	.30	1.71				
Sept. 1	.24	1.27				
Sept. 8	.26	1.47	.38*	1.79*	.44*	1.83*

* Single determination.

The same data are shown graphically in figure 2. The data for half trees collected June 8 are: check, wood .74 per cent, bark 2.20 per cent; defoliated, wood .46 per cent, bark 1.24 per cent. These cover only 1922 material and only a portion of that because of the lack of material. The points indicated by these analyses are:

1. The concentration of reducing sugars is very much greater in the bark than in the wood.

2. At the period covered by these analyses, i.e., from more than a month after growth started until the end of the season, the concentration of reducing sugars has been somewhat reduced in the defoliated series, as compared with the check. It is unfortunate that the material for those collections made immediately after defoliation was exhausted before determinations for reducing sugar were made. The fact that some time had elapsed after defoliation makes the significance doubtful. However, the California material indicates that the reduction in amount takes place very soon after defoliation.

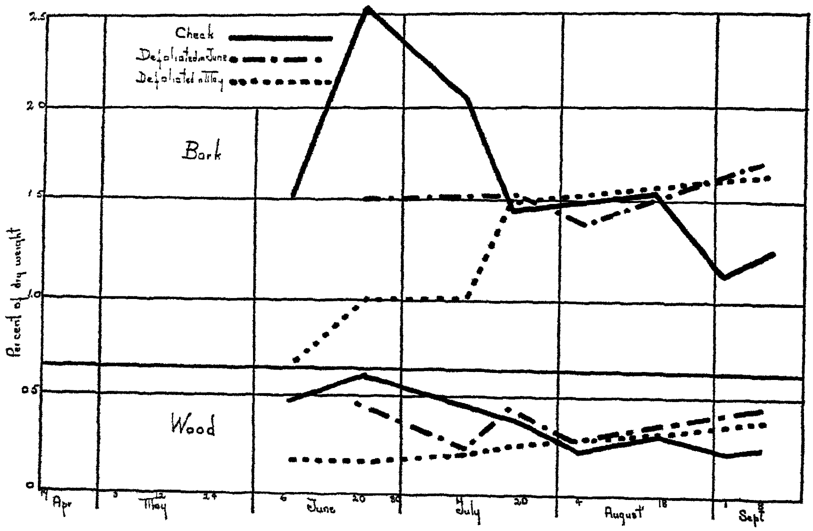

Fig. 2. Effect of defoliation on reducing sugar content of one-year-old twigs of McIntosh apple.

3. The reduction in reducing sugars that is noticed does not appear to be sufficient to account for the cessation of cambial activity, which occurred in every case of the 1922 series before the analyses were made. It would seem that the concentrations indicated are adequate for growth, from the data that are available on this point.

4. Considered with the data for total sugars and starch presented below, there is the suggestion that enzyme activity has been retarded. A partial inactivation of enzymes might account for the phenomena noted.

5. There are fluctuations of considerable magnitude between different collections. These seem to be readily accounted for only by the assumption of differences of this magnitude between different trees.

2. *Total Sugars.*

The data for total sugars are somewhat more extensive than are those for reducing sugars. The results of the analyses for the same material are given to table 11.

TABLE 11

EFFECT OF DEFOLIATION ON THE TOTAL SUGAR CONTENT OF ONE-YEAR-OLD TWIGS OF MCINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Per cent of dry weight as dextrose					
	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
Apr. 19	.68	3.35				
May 3	.59	1.86				
May 12	.79	6.15	1.18*	6.05		
May 24	.95	4.14	.77	4.34		
June 6	1.00	3.98	.65	3 42		
June 20	.73	3.63	.45	2.55*	.79	2.49
June 30	.65	3.03	.43	1 76	.84	1.95
July 11	.67	2.84	.37	1.87	.42	1.76
July 20	.40	2.54	.34	2.13	.44	2.67
Aug. 4	.35	1.95			.38	1.04
Aug. 18	.38	1.97				
Sept. 1	.27	2.21				
Sept. 8	.35	2.09	.58*	2.58*	.60*	2.34*

* Single determination.

The data are shown graphically in figure 3. The results, as in the preceding case, are based on the percentage of dry weight, expressed as dextrose. For the June 8 collection of halves of trees, the percentages are: Check, wood 1.25, bark 6.46; defoliated, wood 1.91, bark 4.22. The results indicate about the same things as do reducing sugars. There is the same marked difference in concentration between wood and bark. There is a better basis of comparison between check and treated series through a more nearly unbroken series of determinations. The differences are not so marked as in the case of reducing sugars. At the beginning of the season, when growth was stopping in the first series defoliated, there was as much total sugar in one as in the other. Fluctuations are less also between collections. There is thus an even smaller suggestion than in the case of reducing sugars that the sugar fraction, at least as a group, is the limiting factor under the conditions of this experiment.

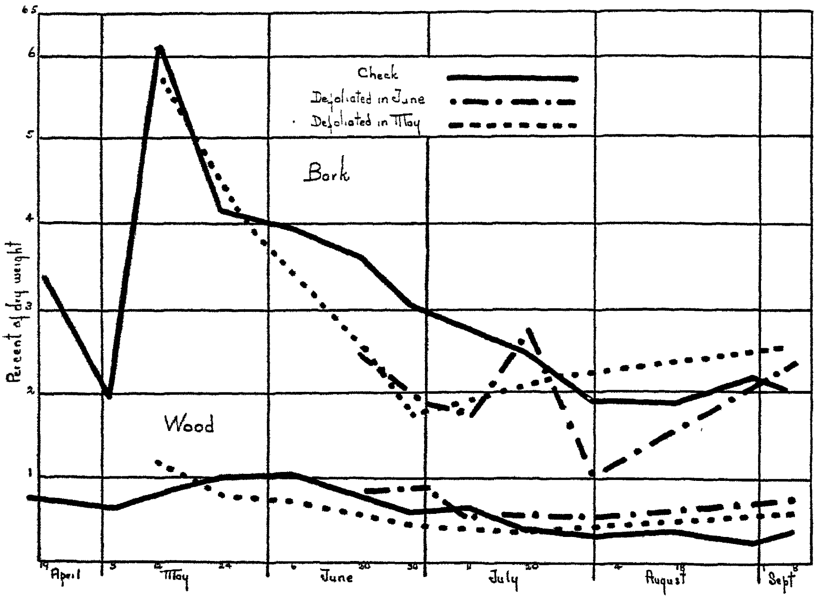

Fig. 3. Effect of defoliation on total sugar content of one-year-old twigs of McIntosh apple.

3. *Starch.*

The sequence of changes in the starch content is interesting from the point of view both of changes in the normally functioning plant, and of comparison with defoliated specimens. Table 12, and figures 4 and 5 give these data expressed as starch (reducing sugar x .9) in per cent dry weight. For the June 8 collection from halves of trees, the percentages are: Check, wood 5.94, bark 10.11; defoliated, wood 7.26, bark 11.93. The normal sequence is similar in most respects to the curves published by numerous workers in the past. There is the usual high initial concentration followed first by a drop almost to zero and then by a gradual rise throughout the remainder of the season. The most striking thing about the analyses is the enormous fluctuation in the bark between successive collections. There is apparently a very high starch deposition in the cortical region of the bark, forming a considerable share of the reserve. As was noted in the case of reducing sugar, there is a suggestion of inactivation of diastase.

The starch disappears more slowly from the defoliated series than from the check. This gives additional evidence, however, that it is not a lack of carbohydrates that is the limiting factor in the checking of growth.

TABLE 12

EFFECT OF DEFOLIATION ON THE STARCH CONTENT OF ONE-YEAR-OLD TWIGS OF THE McINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Per cent dry weight as dextrose x .9					
	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
Apr. 19	4.87	6.95				
May 3	9.28	5.53				
May 12	1.16	6.34	3.21	5.68		
May 24	1.58	2.11	2.39	2.38		
June 6	2.43	5.60	2.94	5.36		
June 20	2.92	2.97	1.48	2.27	1.35	1.60
June 30	3.97	6.11	2.45	6.35	2.48	4.63
July 11	3.06	3.74	1.80	2.10	1.71	1.98
July 20	6.26	5.38	1.31	2.78	1.50	2.53
Aug. 4	3.06	6.20			1.60	2.16
Aug. 18	4.62	9.07				
Sept. 1	6.15	10.23				
Sept. 8	5.54	10.24	2.84	5.01	2.00	3.69

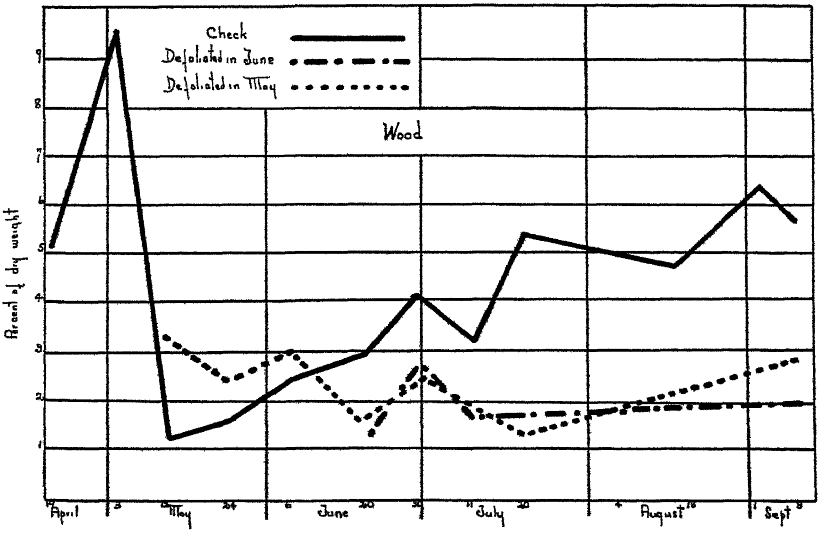

Fig. 4. Effect of defoliation on starch content of the wood of one-year-old twigs of McIntosh apple.

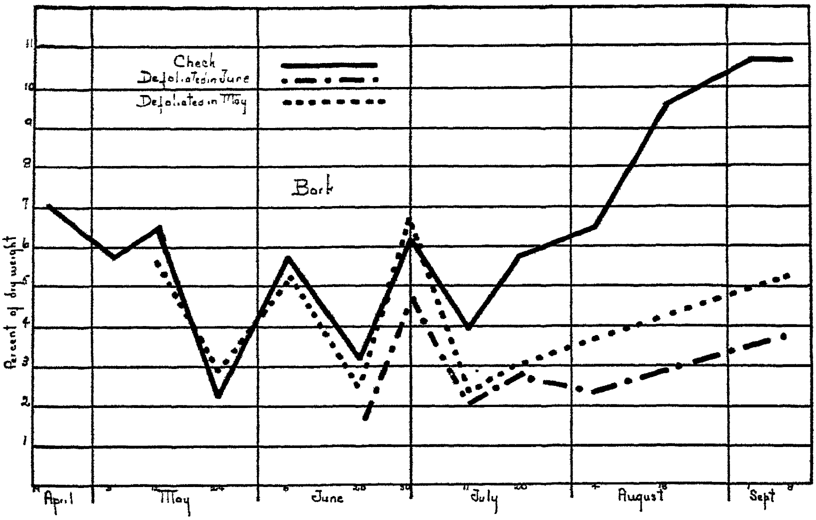

Fig. 5. Effect of defoliation on starch content of the bark of one-year-old twigs of McIntosh apple.

4. *Hemi-Celluloses.*

The analyses for hemi-cellulose (table 13 and fig. 6) show nothing. The half tree analyses are again in accord, June 8 halves given: Check, wood 22.98, bark 10.20; defoliated, wood 24.33, bark 10.69. The data again are expressed as dextrose x .9 in per cent dry weight. There are fluctuations, but they seem to be slight and of no significance. This series was early discontinued. If hemi-cellulose can be utilized as a reserve food, it seems not to have been in the case of

TABLE 13

EFFECT OF DEFOLIATION ON THE HEMI-CELLULOSE CONTENT OF ONE-YEAR-OLD TWIGS OF McINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Per cent dry weight as dextrose x .9					
	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
May 3	19.10	12.81				
May 12	20.24	12.14	20.18	11.55		
May 24	20.88	13.87	21.46	12.37		
June 6	18.47	11.52	20.03	13.00		
June 20	19.02	11.42	17.98	11.32	18.81	12.14
June 30	19.30		20.45			
July 11	19.41	12.06	19.79	11.83	18.12	12.28

this material. There certainly is nothing here to indicate carbohydrate deficiency. A point of interest, however, is the low content in the bark as compared to that of the wood.

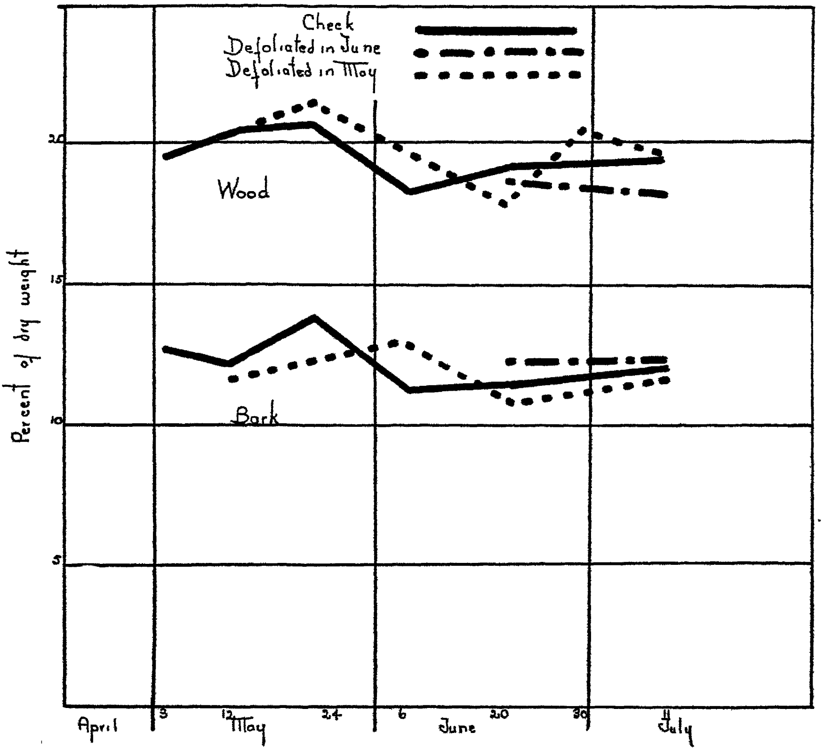

Fig. 6. Effect of defoliation on hemi-cellulose content of one-year-old twigs of McIntosh apple.

5. *Total Nitrogen.*

Finally, there are the analyses for total nitrogen expressed in per cent dry weight (table 14 and fig. 7). A large difference in the nitrogen content between the wood and bark is shown, in the same direction as in the case of the sugars. The total nitrogen content of the check series falls off steadily throughout the summer. The defoliated series, however, shows no such falling off. In the bark there is a gradual increase, and in the wood a slight decrease. The series defoliated in June, while not showing the rise seen in the first series, does in the bark show a distinct check in the rate at which it falls off and maintains a generally higher level than does the check. The wood, with a much smaller content, is not so regular. There seems to be much less fluctuation between trees than is the case with the carbohydrates. There is no suggestion whatever that total nitrogen is a limiting factor.

TABLE 14

EFFECT OF DEFOLIATION ON THE TOTAL NITROGEN CONTENT OF ONE-YEAR-OLD TWIGS OF THE MCINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Per cent dry weight					
	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
Apr. 19		1.19*				
May 3	.51*	1.24				
May 12	.53	1.00	.59*	1.08		
May 24	.51	.91	.69	1.19		
June 6	.34	.92	.40	1.17		
June 20	.43	.73	.61	1.29	.38	1.18
June 30	.40*	.88	. .	1.61	...	1.04
July 11	.42	1.02	.48	1.37	.42	1.13
July 20	.14	.59	.33	1.16	.23	.84
Aug. 4	.10	.62			.13*	.83*
Aug. 18	.18	.60				
Sept. 1	.18	.56				
Sept. 8	.27	.67	.42	1.23	.20	.82
For halves of trees						
June 8	.30	.80	.36	1.04		
June 28	.20	.67	.30	.90		
June 26	.21	.67			.23	.68
Sept. 10	.14	.62			.16*	.67*

* Single determination

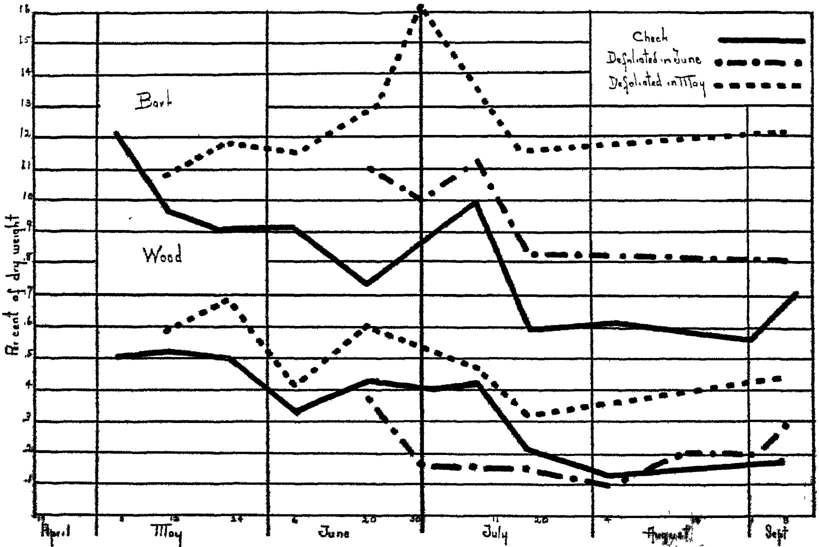

Fig. 7. Effect of defoliation on total nitrogen content of one-year-old twigs of McIntosh apple.

6. *Carbohydrate-Nitrogen Ratio.*

The only thing remaining to be done with these data, then, is to determine whether or not the carbohydrate-nitrogen ratio, or rather the starch-nitrogen or the sugar-nitrogen ratio, can be presented as a cause for the phenomenon. Table 15 shows the starch-nitrogen ratio for the collections up to and including July 20. Generally there is a higher ratio in the wood than in the bark. This is, of course, what would be expected. The ratio is uniformly lower in the bark of the defoliated trees than in that of the check.

TABLE 15

EFFECT OF DEFOLIATION ON STARCH–NITROGEN RATIOS IN ONE–YEAR–OLD TWIGS OF McINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
May 3	18.2	4.4				
May 12	2.2	6.4	5.4	5.3		
May 24	3.1	2.3	3.5	2.0		
June 6	7.1	6.1	7.3	4.6		
June 20	6.7	4.0	2.4	1.8	3.6	1.4
June 30	9.9	7.0		3.9		4.4
July 11	7.4	3.7	3.8	1.5	4.1	1.7
Halves of trees						
June 8	19.5	12.5	20.0	11.5		

The ratio of total sugar to nitrogen (shown in table 16) was calculated for the same material used for table 15. The ratios found for wood are again inconclusive. Those for bark are more consistent than were those in table 15, and all deviate in the same direction as do those in table 15, that is, the sugar-nitrogen ratio is less in the bark of defoliated series than in the corresponding checks.

As has alrealy been indicated, the analyses of whole twigs are given little weight in this discussion. Therefore, the writer will be content to present the data in table 17 for the McIntosh orchard for 1923 and table 18 for the greenhouse-grown seedlings with little comment. The former seem to show a slightly greater reduction in carbohydrates than was found in the previous season's analyses, but in general substantiate the earlier results. As was to be expected, the variability in composition of the seedlings is very much greater than in the other series. There are no seriously conflicting data. The series having the buds removed gave the most striking results, as it did in the growth measurements. It indicates, perhaps, that the difference in carbohydrates is more to be associated with the amount of material used in pushing out new length growth than with cambial activity. This would account for the fact that this series shows such a slight reduction in carbohydrates as compared with those series which were allowed to form terminal growing points.

TABLE 16

EFFECT OF DEFOLIATION ON THE SUGAR-NITROGEN RATIOS IN ONE-YEAR-OLD TWIGS OF THE MCINTOSH APPLE, 1922. WHOLE TREES TREATED

Date	Check		Defoliated in May		Defoliated in June	
	Wood	Bark	Wood	Bark	Wood	Bark
May 3	1.1	1.1				
May 12	1.5	6.1	2.0	5.7		
May 24	1.9	4.6	1.1	3.6		
June 6	3.0	4.3	1.6	2.9		
June 20	1.7	4.9	.7	2.0	2.1	2.2
June 30	1.6	3.5		1.1		1.9
July 11	1.6	3.8	.8	1.4	1.0	1.5
Halves of trees						
June 8	4.2	8.1	2.5	4.0		

TABLE 17

EFFECT OF DEFOLIATION ON THE CHEMICAL COMPOSITION OF ONE-YEAR-OLD TWIGS OF THE MCINTOSH APPLE, 1923. WHOLE TREES TREATED. WHOLE TWIGS ANALYZED

Date	Reducing sugars % dry weight		Total sugars % dry weight		Starch % dry weight		Total nitrogen % dry weight	
	Check	Defoliated	Check	Defoliated	Check	Defoliated	Check	Defoliated
May 4	1.81		1.91		4.44*		.73*	
May 28	1.28	.99*	1.18	.94*	.76	.72	.52*	.61*
June 12	1.60	.79	1.78*	.89	1.35	.93	.52	.63

* Single determinations.

TABLE 18

EFFECT OF DEFOLIATION AND DISBUDDING ON THE CHEMICAL CONTENT OF ONE-YEAR-OLD SEEDLINGS, 1923. WHOLE TWIGS USED FOR ANALYSIS. PER CENT DRY WEIGHT

Date	Treatment*	Reducing sugar % dry weight	Total sugar % dry weight	Starch % dry weight	Total nitrogen % dry weight
May 7........	1	.79	95	1.85	.56
	2	.48†	.63†	.87	.32†
	7	.68†		2.83†	.70†
May 25	1	.99	1.11	2.42	.55
	5	.52		.83	.53
	6		1.18	.60	.50
May 30	2				.51†
	7				.77†
June 7...............	1	.73	1.05	3.04	.54
	2	.35†		1.30	.67
	3	.55†	1.18†	.74	.65†
	4				.45†
	5				.52†
	6				.59†

* Treatment—1—Check.
—2—Defoliated throughout.
—3—Defoliated after April 13.
—4—Defoliated until April 13, then allowed to grow.
—5—The same as 4 until May 15, then defoliated again.
—6—The same as 3 until May 15, then allowed to grow again.
—7—Disbudded.

† Single determinations.

TABLE 19

THE EFFECT OF DEFOLIATION AND DISBUDDING ON THE REDUCING SUGAR CONTENT OF ONE-YEAR-OLD TWIGS OF RED ASTRACHAN APPLE, 1924. HALF TREES TREATED

Date	Check		Defoliated		Date	Check		Disbudded	
	Wood	Bark	Wood	Bark		Wood	Bark	Wood	Bark
Mar. 7	.98	2.46							
Mar. 15.......	.94	1.81							
Mar. 20......	1.01	2.30	1.00	1.69	Mar. 18.......	.99	1 49	.79	1.59
Mar. 25 .	.81	2.05	.57	1.58	Mar. 25	1.02	1.68	.69	1.75
Apr. 8.........	1.54	2.30	1.24	2.04					
Apr. 22...... .	1.13	2.02	.87	2.45					

TABLE 20

THE EFFECT OF DEFOLIATION AND DISBUDDING ON THE TOTAL SUGAR CONTENT OF ONE–YEAR–OLD TWIGS OF RED ASTRACHAN APPLE, 1924. PER CENT DRY WEIGHT. HALF TREES TREATED

Date	Check		Defoliated		Date	Check		Disbudded	
	Wood	Bark	Wood	Bark		Wood	Bark	Wood	Bark
Mar. 7	1.07	2.72							
Mar. 15	1.18	3.71							
Mar. 20		3.15		2.63	Mar. 18	1.08	4.45	.94	4.18
Mar. 25	.97	4.89	.68	4.59	Mar. 25	1.51	4.77	1.24	4.62
Apr. 8	2.41	5.67	2.18	6.54					
Apr. 22	1.83	5.89	1.35	5.26					

TABLE 21

THE EFFECT OF DEFOLIATION AND DISBUDDING ON THE STARCH CONTENT OF ONE–YEAR–OLD TWIGS OF RED ASTRACHAN APPLE, 1924. HALF TREES TREATED. PER CENT DRY WEIGHT

Date	Check		Defoliated		Date	Check		Disbudded	
	Wood	Bark	Wood	Bark		Wood	Bark	Wood	Bark
Mar. 7	2.84	3.20							
Mar. 15	2.62	2.52							
Mar. 20	2.48	2.56	3.09	3.40	Mar. 18	2.26	2.54	2.39	2.42
Mar. 25	2.06	1.52	1.93	1.90	Mar. 25	2.12	1.26	2.15	1.62
Apr. 8	1.11	1.02	1.13	1.25					
Apr. 22	0.00	.77	0.00	.77					

TABLE 22

THE EFFECT OF DEFOLIATION AND DISBUDDING ON THE TOTAL NITROGEN CONTENT OF ONE–YEAR–OLD TWIGS OF RED ASTRACHAN APPLE, 1924. PER CENT DRY WEIGHT. HALF TREES TREATED

Date	Check		Defoliated		Date	Check		Disbudded	
	Wood	Bark	Wood	Bark		Wood	Bark	Wood	Bark
Mar. 7	.50	.94							
Mar. 15	.51	.95							
Mar. 20	.48	.94	.50	.98	Mar. 18	.45	.91	.49	.92
Mar. 25	.47	.80	.50	.88	Mar. 25	.43	.87	.42	.93
Apr. 8	.35	.74	.45	.80					
Apr. 22	.48	.74	.43	.78					

DISCUSSION AND CONCLUSIONS

The actual factor limiting growth under conditions of defoliation has been a matter of speculation by a number of writers. Harper[4] has summarized the theories heretofore brought forward as follows:

1. Wieler and Hartig suggest lack of food.

2. Schwarz, lack of pressure from bending movements.

3 Strasburger and Haberlandt, the need of the plant for an increased water supply.

4. Lutz, an excess of water in the tissues due to decreased transpiration. Harper himself favors the first idea, though he presents nothing to substantiate the suggestion.

Harvey[5] has added another in the form of the carbohydrate-nitrogen ratio.

Roberts[12] suggested lack of nitrogen, a phase of the first.

Considering these suggestions, it seems from the data presented by Harvey and by the writer that the lack of total nitrogen is not the factor sought under these conditions. The fact that Roberts' work was done at a different time of year, and with different material may explain the discrepancy.

The starch-nitrogen ratios calculated for this material do not in the writer's estimation, adequately account for the observed results. The ratio is subject to such wide fluctuations and the differences between the series are generally so slight that to assign this as the controlling factor in limiting growth seems to be an unwarranted assumption. Although sugar-nitrogen is less variable, at least in the bark, the differences do not appear to be so great at first as later, and this ratio also seems not to be the limiting factor, though a definite conclusion cannot well be drawn. The possibility still remains that it is not total nitrogen, but some fraction of it which may be the essential thing in the ratio, but there are insufficient data on this point to warrant a discussion of the idea. The fact that Nightingale[9] has found it necessary to modify Kraus's original postulates along this line may perhaps lend color to the suggestion.

Harvey's data on the moisture content of the different series may seem to furnish a basis for Lutz' proposal. It seems that larger differences in water content would be necessary to effect such radical changes in the metabolism of the plant as those observed, especially in view of the fact that differences between the bases and tips of a series of a given treatment were generally greater than between series of different treatments. Perhaps these differences can be largely attributed to differing proportions of wood and bark, since the proportion of wood is much greater in the base than in the tip. That water pressure may delay growth has been pointed out by Heinicke.[6] though the differences in moisture content that accompanied this phenomenon are not given. Moisture content is of doubtful significance at best, and it is questionable whether this suggestion can be given much weight.

The least acceptable theory given above is that of ''the need of the tree for an increased water supply.'' The data available indicate an excess of water rather than a deficiency, while at the same time the ''need'' for it is reduced by the lack of leaf surface. These considerations, in addition to the natural reaction against a teleological explanation, dispose of this idea.

Schwarz' hypothesis does not seem to have been very well received by recent investigators. It may be said to have received some support from the distribution of xylem in the annual rings of trees growing in a region of strong winds that are uniformly from one direction. However, the fact that plants develop without this stimulus under greenhouse conditions indicates that it is probably a minor factor.

Neither the analyses of Harvey nor those here given are of much value in supporting the idea of starvation. It still remains a possibility that starvation, due to the lack of some specific compound, either a fraction of one of the groups treated or some unrelated compound, may be the true explanation of the condition.

A closely related idea is that of growth promoting substances. It has been suggested that many activities of the plant are regulated by hormones. If these are produced by the leaves, the supply might be cut off by defoliation. This idea is highly speculative, however.

The hypothesis that the presence of inhibitory substances, perhaps of the nature of the ''staling'' substance of fungous cultures, may be responsible, is also speculative. It might be assumed that the leaves are effective in removing or counteracting the effect of such substances.

A more promising hypothesis, and one suggested by the data, is the partial or complete inactivation of enzyme activity. It has already been suggested that such an assumption is in accord with the relations observed between the various carbohydrates. This assumed inactivation might be brought about by a change in the hydrogen-ion concentration of the cell sap, by the failure of the zymogen to change to the active enzyme, or by the formation of compounds between the enzyme and other substances in the cell, perhaps the substrate, with subsequent failure to disjoin. A change in hydrogen-ion concentration, again, might have other effects than that indicated above. The most obvious is a change in the proteins, as indicated by the work of Loeb, either by precipitation of the protein at the iso-electric point, or the change from one type of compound to another. The strong buffer action of cell sap does not support this idea.

The localization of certain compounds in certain tissues, as indicated by the difference in wood and bark, may be carried much farther. That is, the meristematic region may be suffering from a lack of certain foods while a closely adjacent tissue may have an abundance of the substance, and through the failure of lateral translocation, be unable to supply it to the point where it is needed for growth. This is further suggested by the fact that terminal meristems are able to continue growth long after the lateral one has ceased to function. The analysis of a group of tissues such as those present in the bark would effectually mask the relations between them.

The possibilities outlined above indicate that there is nothing that can be legitimately concluded as to the relation of foods to the activity of the cambium except that totals of the groups above indicated are not the limiting factors. The indications point away from starvation, but do not eliminate it. The most plausible hypotheses appear to the writer to be:

1. Enzyme inactivation, with consequent lack of certain end products of digestion necessary for growth.
2. Starvation, due to failure of the leaves to supply certain compounds directly, or to failure of translocation through short distances.
3. Lack of balance of carbohydrate-nitrogen ratio, calculated not on totals but on fractions that may prove to be more directly involved.

SUMMARY

1. Defoliated apple trees, or halves of trees, showed a cessation of radial increase of wood within two weeks after defoliation.
2. This was accompanied by a modification of the thickness of the walls of the cells laid down after defoliation.
3. This phenomenon does not seem to be associated with a deficiency of stored food as indicated by analyses for reducing sugar, total sugar, starch, hemicellulose, and total nitrogen in the wood and in the bark.
4. There are several alternate theories that might be suggested to account for the phenomena.

ACKNOWLEDGMENTS

The subject of this paper was suggested by Dr. Lewis Knudson, under whose direction the work was carried on, and to whom the writer is indebted for valuable advice and suggestions.

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THE UTILIZATION OF SULFUR DIOXIDE IN THE MARKETING OF GRAPES

A. J. WINKLER AND H. E. JACOB

The rapidly increasing volume of fresh grapes being shipped each year from California often taxes the resources of the railroads severely and during much of the season of 1922 resulted in serious congestion. The condition was similar, though less intense, in 1923. As a result, large quantities of grapes could not be shipped and were left to rot on the vines, while in those shipped, losses from spoiling were much increased by delays in delivery.

In consequence of these losses many inquiries were sent to the College of Agriculture as to methods of bettering these conditions either by improving the efficiency of the refrigerator cars now in use or by employing other modes of transportation.

Several possibilities suggested themselves, all based on the idea of delaying the deterioration of the grapes. This might make it possible to spread the shipments over a longer season, to utilize cars without refrigeration, and to increase the efficiency of refrigerator cars. The capacity of the railroads with their present equipment would thus be increased.

The causes of deterioration in transit or in storage are chiefly evaporation of water from the grapes and consequent shrivelling, and the activity of various micro-organisms causing decay. While on the vine, the grapes are freely exposed to dry air which retards the growth of the micro-organisms, and the water lost by transpiration is replaced by the vine. After removal from the vine, however, the grapes must be prevented from shrivelling by being kept in a relatively moist air to prevent evaporation. Moist air, however, fosters the growth and activity of micro-organisms. The growth of these organisms can be controlled by sterilization, refrigeration, or chemical preservatives. By sterilization, as in ordinary canning, the conditions of texture, color, and flavor are changed so that the product is no longer fresh fruit. By refrigeration, it is possible to retain the fresh flavor, color, texture, etc., but the time that grapes can be held in this way is limited. Although little is known of the possibility of retarding deterioration of grapes by means of chemical preservatives, the nature of some of these preservatives, together with the positive results they have given in the control of saprogenic (decay causing) organisms in the manufacture of certain fruit products such as cider, grape juice and wines, indicate that it might be possible to prevent the spoiling of fresh grapes for a considerable period by their use.

OBJECT OF THE INVESTIGATION

This investigation is an attempt to determine (1) the possibility of preserving grapes fresh in sealed containers by means of preservatives so that they will be suitable for manufacturing purposes for several months after their removal from the vines and (2) the possibility of retarding spoiling under the present system of refrigerator car transportation, by means of chemical preservatives with a view to placing the grapes on the Eastern markets in better condition.

PRELIMINARY TESTS WITH VARIOUS CHEMICALS

Sulfur dioxide, which has been used successfully for many years as a mean of controlling undesirable organisms in wine making, and various other chemical preservatives, some of which have been used rather widely in fruit and vegetable products, were tested.

In the preliminary tests the grapes were submerged in fresh must containing the various preservatives. The results are in agreement with those obtained by Scott and Will.* Sulfur dioxide was not used by them.

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* Scott, R. D., and Will, S. G., Cider Preservatives. Jour. Ind. and Eng. Chem., 13:1141, 1921.

RESULTS OBTAINED WITH VARIOUS PRESERVATIVES

Boric Acid.—In concentrations of from .1 to .15 per cent, boric acid retarded alcoholic fermentation for only a few days. Acetic acid accumulated very rapidly.

Formic Acid.—In the same concentration this acid gave results identical with those obtained with boric acid.

Formaldehyde.—In concentrations of .005 to .0075 per cent, formaldehyde did not retard fermentation.

Benzoate of Soda.—In concentrations of from .1 to .2 per cent, benzoate of soda prevented alcoholic fermentation for several months. The production of acetic acid, which was not inhibited, however, spoiled the grapes in the course of from four to six weeks.

Salicylic Acid.—In concentrations of from .1 to .15 per cent, salicylic acid prevented alcoholic fermentation for from four to six weeks. The production of acetic acid was not retarded.

Sulfur Dioxide.—In concentrations of from .06 to .12 per cent, sulfur dioxide gave indications of controlling both the alcoholic fermentation and the production of acetic acid.

Results very similar to those given above were obtained when the grapes were dipped in solutions of the several preservatives of widely varying concentrations. In these tests again sulfur dioxide alone gave indications of favorable results.

In view of the results obtained in the preliminary tests, all preservatives except sulfur dioxide were soon discarded as unpromising.

SULFUR DIOXIDE (SO_2)

Physical and Chemical Properties.—Sulfur dioxide (SO_2) is a colorless gas, 2.2 times as heavy as air. Its odor, which is very pungent, is characteristic of the fumes produced by burning sulfur. The gas itself will not burn. The gas is soluble in water to the extent of about one part in ten parts of water by weight at room temperature. As a water solution, this substance is known as sulfurous acid. At ordinary atmospheric pressure the gas liquefies at a temperature of —10° C. The liquefied gas is retailed in steel drums, which must be sufficiently strong to withstand considerable pressure, since at 20° C. the gas exerts a pressure of 40.6 pounds to the equare inch.

The gas bleaches organic colors. This action has been widely utilized in the decolorization of walnuts and dried fruits. The gas owes this property to its power of forming colorless compounds by combining with the color chromogens. As a rule, these compounds are easily broken up by oxidation and the color restored. Red grapes can be made white by treatment with SO_2, but on aeration the color returns. In this respect the bleaching action of SO_2 differs from that of other bleaching agents which either destroy or remove the color.

Sulfur dioxide combines readily with certain organic substances found in the grape including the sugars. This combination of SO_2 and sugar is fairly stable and doubtless constitutes a major part of the combined SO_2 in the treated grapes. When grapes are treated with SO_2, there is also a portion of the gas that does not enter into any combination. It is this portion which is usually termed *free* SO_2. It is to the free SO_2 that the retardation or prevention of spoiling is due, since in this form it is a very active preservative.

TABLE 1

THE EFFECT OF SULFUR DIOXIDE ON THE MULTIPLICATION OF MICRO-ORGANISMS

Organism*	No. of cells per c.c. at start	Number of living cells per c c. after 36 hours exposure to the following concentrations.				
		Milligrams of sulfur dioxide per liter				
		None	50	100	200	400
Wine Yeast	20,000	....	640,000	2,000,000	310,000	36,000
Apiculatus	150,000	....	200,000	75,000	56,000	0
Wild Yeast (Pastorianus form.)	620,000	....	580,000	6,000	190	0
Penicillium	120,000	....	40,000	0	0	0
Aspergillus	450,000	....	120,000	20,000	30,000	0
Vinegar Bacteria	310,000	610,000	14,000	300	2	0

* All of these organisms except the wine yeast were isolated from California grapes.

The Retarding Effect on the Growth of Saprogenic Organisms.—Spoiling of grapes in storage or transit is due primarily to the activity of molds, yeasts, and bacteria. Of the molds, types of Penicillium, Aspergillus, Botrytis, Mucor, and Monilia are the most common. Of the yeasts, the true wine yeast (*Saccharomyces ellipsoideus*), the wild yeasts (forms of *S. pastorianus* and *S. apiculatus*), and the pseudo yeasts, Mycodermae, are the most common. The bacteria usually met with are forms producing vinegar.

The growth of all of these organisms is retarded or prevented by SO_2 in sufficient concentration. The effect of different concentrations of SO_2 on the multiplicaton of some of these organisms as observed by Cruess* is shown in table 1. Chabert† found that visible active fermentation of grape must was retarded by the addition of small quantities of sulfur dioxide. His observations are given in table 2.

The Effect on the Metabolism of Grapes.—Sulfur dioxide in addition to controlling or inhibiting the growth and activity of micro-organisms effects a slowing up in metabolic changes, e.g., the respiration which normally occurs in grapes. Tests were made in duplicate with treated and normal grapes in order to determine the rate at which carbon dioxide is liberated. As shown by the graphs in figure 1, the slowing up in the release of carbon dioxide is positive and for amounts less than 300 mgs. per kilo. of grapes is more or less proportional to the amount of sulfur dioxide applied. The figures plotted here were obtained with Grenache which had a sugar content of 26 degrees Balling.

TABLE 2

THE EFFECT OF SULFUR DIOXIDE IN RETARDING VISIBLE FERMENTATION

Concentration of SO_2		Time that visible fermentation was retarded	
Milligrams per liter	Per cent	Ordinary temperature (presumably 20° C.)	28° C.
10	0.001	Appreciably	...
30	0.003	10–12 hours	...
50	0.005	18–21 hours	6 hours
75	0.0075	48–60 hours	...
100	0 01	5–6 days	32 hours
150	0.015	...	97 hours
200	0 02	...	146 hours
250	0.025	...	More than 8 days

It is of interest to note the uniform slope of the several graphs in figure 1. The effect of the SO_2 seems to be confined to the life processes of the grape with little or no destruction of the tissue. The variations in the individual determinations were a result of the fluctuating temperature in the laboratory.

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* Cruess, W. V., The effect of H_2SO_3 on fermentation organisms. Jour. Ind. and Eng. Chem., 4:581–585, 1912.

† Chabert, F., Prog. Agr., 37:574–579, 1892.

SULFUR DIOXIDE IN THE PRESERVATION OF GRAPES IN SEALED CONTAINERS*

The object in mind in making these tests was that of devising means, if possible, whereby grapes intended for grape juice or other grape products could be packed and preserved for temporary storage or shipment without refrigeration and without elaborate equipment or great expense. A method capable of this would not only enable the growers of grapes for manufacturing purposes to dispose of their entire crop more readily, but might also release a considerable number of refrigerator cars for the handling of our increasing tonnage of table grapes.

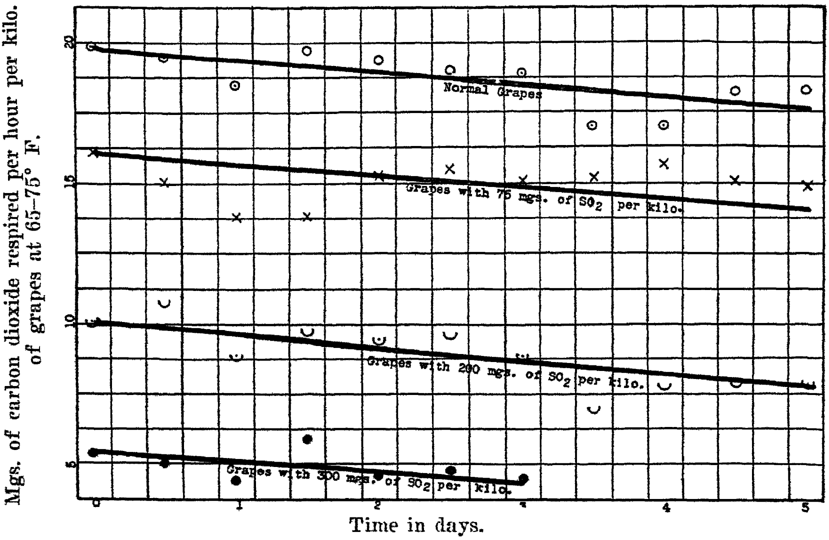

Fig. 1. The effect of SO_2 on the rate of respiration in grapes.

Sulfur dioxide has been used for years to hold crushed grapes (must) in temporary storage during the rush season of harvesting.

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* Some tests were made by F. T. Bioletti on the preservation of fresh grapes in sealed containers containing atmospheres of SO_2, CO_2, alcohol vapor and air. These were reported in the Report of the Vit. Work of the Coll. of Agr., Univ. of Calif., 1896, 447–450.

Some preliminary tests on the shipping of grapes in closed barrels were reported by F. T. Bioletti in the California Grape Grower, vol. 5, August, 1924. In these tests the Grape Growers Exchange, the Sun Maid Raisin Growers Association, and the California Barrel Company were coöperating with the College of Agriculture of the University of California.

In European countries it has also been used to retard fermentation in short distance shipments of must and grapes from the vineyards to the wineries. Impetus was given to these tests by the landing in New York of Italian grapes in barrels during the latter part of the 1922 season. Although not entirely successful, these shipments of Italian grapes indicated that, with a sufficient knowledge of all the factors concerned, the sugar content of grapes might be retained almost indefinitely.

TABLE 3

THE RETARDING EFFECT OF SULFUR DIOXIDE ON THE SPOILING OF GRAPES IN SEALED CONTAINERS AS INDICATED BY SUGAR CONTENT

Variety	Mgs. of SO_2 per kilo. of grapes	Balling degree of the expressed juice						
		At the beginning	After 1 month	After 2 months	After 3 months	After 4 months	After 5 months	After 8 months
Series A								
Valdepeñas.....	0	23.	0 After 10 days					
	350*	23.	0 After 20 days					
Alicante Bouschet	0	22.5	0	....	...	... .	... .	
	300*	22.5	15 7	... .	....	... .	...	
	300*	22.5	17.5	3 8	0 1	... .	. . . . .	
	600*	22.5	19.2	18.8	15.4	17.5	... .	
Malaga	0	22.5	0	. . .	. ....	. ....	. .	
	600*	22.5	19.6	18.4	. ..	... .	21 2	
Series B								
Cornichon..........	0	18.4	0	....	... .	.. ..	....	. .. .
	600†	18.4	17.6	18.1	. ..	... .	18.7	4.5
	900†	18.4	....	... ...	. .. .	. . . .	19.8	14.4
	650‡	18.4	16.9	16.5	.. ....	.. . .	19.3	....
	900‡	18.4	....	.... .	18 6	. .. .	20.4	. . ..
	650§	19.0	16.2	16.4	... ..	1.5	.. ..	....
Series C								
Tokay	0	20.4	0	. . ....	....	. .. . ..	....	
	900†	20.4	20.1	19.0	20 8	20.3	20.8	
	650†	20.8	18.7	18.6	20.7	20.4	20.3	
	550†	20.5	18.4	18.4	19 4	19.5	19.5	
	800†	21.3	22.1	21.6	21.6	20.4	20.7	

* SO_2 applied in a small amount of water.
† SO_2 applied by immersion.
‡ SO_2 applied as gas.
§ SO_2 applied in must.

Retardation of Spoiling.—Although it is recognized that the free rather than the combined SO_2 is primarily responsible for the effect on micro-organisms, only the total SO_2 present in the grapes will be considered in this discussion, since it is obviously impossible to control the relative amounts of free and combined SO_2 in the filled containers. It should, however, be borne in mind that there is a more or less direct relation between the free and the total amount of SO_2 present.

TABLE 4

THE RETARDING EFFECT OF SULFUR DIOXIDE ON THE SPOILING OF GRAPES IN SEALED CONTAINERS AS INDICATED BY THE ACCUMULATION OF ALCOHOL

Variety	Mgs. of SO_2 per kilo. of grapes	Per cent of alcohol present						
		At the beginning	After 1 month	After 2 months	After 3 months	After 4 months	After 5 months	After month8
Series A								
Valdepeñas........	0	Trace	Mold	y afte	r 10 d	ays		
	350	Trace	Mold	y afte	r 10 d	ays		
Alicante Bouschet ..	0	Trace	11.55	....	....	....		
	300	Trace	1.92	9.0	....	....		
	300	Trace	1.02	8.8	9.1	......		
	600	Trace	0.35	0.75	0 75	0.83		
Malaga........	0	Trace	9.1	....	......	....	....	
	600	Trace	Trace	Trace	....	....	0.2	
Series B								
Cornichon........	0	Trace	7.8	....	....	....	....	....
	600	Trace	0.17	0.19	....	....	0.29	6.2
	900	Trace	....	....	....	....	0.2	1.1
	650	Trace	0.23	0.37	....	....	0.19	....
	900	Trace	....	....	0.18	....	0.27	....
	600	Trace	0.25	0.18	....	7.5	....	....
Series C								
Tokay........	0	0.25	Com	pletel	y spoi	led aft	er 13	days
	900	0.3	0.3	0.3	0.3	0.3	0.3	
	650	0.2	0.2	0.2	0.2	0.2	0.2	
	550	0.2	0.2	0.2	0.2	0.2	0.4	
	800	0.25	0.3	0.3	0.3	0.2	0.3	

To obtain data on the efficiency with which spoiling could be controlled in grapes in sealed containers, several series of jars, kegs, and barrels filled with grapes were treated with varying amounts of SO_2. The amount and manner of applying the SO_2 and the results from representative containers with regard to loss in sugar content and the accumulation of alcohol are given in tables 3 and 4.

An examination of tables 3 and 4 reveals that 300–400 mgs. of SO_2 per kilo. of grapes is insufficient to successfully retard the spoiling of grapes in sealed containers. Although spoiling was retarded from four to six weeks in some containers, others under similar conditions kept little better than the untreated checks. This wide variation in the results obtained in these tests indicates that 300–400 mgs. is below the minimum required for practical purposes to preserve grapes.

By increasing the amount of SO_2 to 600 mgs. per kilo., it was possible to retain nearly the entire sugar content of the grapes and to suppress the accumulation of alcohol for from four to six months. The efficiency with which SO_2 at this concentration preserved the grapes in sealed containers is best illustrated by the results obtained under series B and C as shown in the above tables. These series were carried out after some of the difficulties which resulted in early spoiling, e.g., lack of uniformity in the distribution of the SO_2, leaky containers, etc., in series A, had been discovered and special efforts made to overcome them.

Again, by increasing the amount of SO_2 to 800–900 mgs. per kilo. it appears that the loss of sugar and the accumulation of alcohol may be retarded almost indefinitely. At present (March, 1925) grapes have been held for eight months under these treatments with little or no variation in the sugar and alcohol contents.

Importance of Uniform Distribution.—The results of the first tests made with SO_2 in sealed containers varied greatly. Analysis of the methods used in applying the preservative and of the results obtained indicated that this variation in the behavior of lots treated in a similar manner was due chiefly to lack of uniform distribution of the preservative throughout the grapes.

Table 5 shows the behavior of three representative units of each of three series. One unit, barrel No. N. 39, treated with approximately 300 mgs. SO_2 per kilo. of grapes still gave a sugar test of 11 degrees Balling six months after treatment; while another unit, barrel No. N. 44, treated with 600 mgs. per kilo., showed practically no sugar at the end of six months. The entire "D" series of Malaga grapes, three representative units of which are shown in table 5, treated with approximately 600 mgs. of SO_2, gave very uniform results.

Table 6 shows an analysis of one of each of these three series. The two series of Alicante Bouschet represented by barrels Nos. N. 33, 34, and 39, and N. 43, 44, and 49 treated with 300 mgs. and

TABLE 5

VARIATION IN THE BEHAVIOR OF VARIOUS BARRELS

Barrel	Size of barrel in gallons	Variety	Approximate SO_2 added in mgs. per kilo.	Period of storage	Balling degree
N. 33	10	Alicante Bouschet.	300	1 month	15.7
N. 34	10	Alicante Bouschet.	300	6 months.	0.
N. 39	10	Alicante Bouschet.	300	6 months.	11.0
N. 43	10	Alicante Bouschet	600	1 month	19 4
N. 44	10	Alicante Bouschet	600	6 months.	0 5
N. 49	10	Alicante Bouschet	600	6 months.	13.5
D. 9	10	Malaga	600	1 month	19.6
D. 10	10	Malaga	600	2 months.	18.4
D. 11	10	Malaga	600	6 months.	21.2

TABLE 6

VARIATIONS IN DIFFERENT PARTS OF THE SAME BARREL

	SO_2 content mgs. per kilo.	Balling degree	Alcohol content in per cent
Keg Number N. 33, Alicante Bouschet after 1 month:			
Grapes near top of keg	164	19.7	0.95
Grapes near center of keg	45	14.5	2.96
Grapes near bottom of keg	365	18.5	0 98
Keg Number N. 43, Alicante Bouschet after 1 month:			
Grapes near top of keg	393	22.8	0 26
Grapes near center of keg	75	17.3	1.2
Grapes near bottom of keg	470	18.8	0 21
Keg Number D. 11, Malaga after 6 months:			
Grapes near top of keg	395	19.8	0.28
Grapes near center of keg	384	22 0	0.21
Grapes near bottom of keg	574	21.7	0.13

600 mgs. SO_2 respectively show a relatively high concentration of SO_2 at the bottom of the barrel, a lower concentration at the top and a very low concentration at the center of the barrel. Because of this low concentration of SO_2 in the center of the barrel there was a correspondingly low sugar and high alcohol content in grapes in this part of the container. Barrel No. D. 11, representing a series of Malaga treated with approximately 600 mgs. of SO_2 per kilo., shows a very

much more uniform distribution of SO_2 throughout the grapes and after six months the sugar content and alcohol content were practically the same as when treated. To prevent spoiling, the concentration of SO_2 in the grapes in all parts of the container must be sufficiently high to prevent the growth of micro-organisms.

Relation of Type and Size of Container to Efficiency.—In these tests three types of containers were used:

1. Glass containers of several sizes and several methods of sealing.
2. Metal cans of about 5 gal. capacity. These cans were coated with paraffin on the inside to prevent corrosion of the metal by the acids of the grapes and the SO_2.
3. Wooden kegs and barrels. These were of four sizes, 4 gal., 10 gal., 25 gal., and 50 gal. Some were plain fir kegs, some were paraffin lined, some asphalt lined and others were coated with paraffin on the outside after filling.

The behavior of the grapes in these various types and sizes of containers was comparable so long as the distribution of the SO_2 was uniform throughout the grapes in the container, and so long as the container itself remained air tight. Difficulty was experienced with the drying out of the plain wood kegs so that they ceased to be air tight, but except for this there was no difference in any of the experiments that could be attributed to the type or size of the container. Under similar conditions with similar uniform concentration of SO_2 the grapes kept equally well in 50-gallon wooden barrels and 2-quart glass preserving jars.

Relation of the Form of the Sulfur Dioxide to Efficiency.—The sulfur dioxide was applied to the grapes in three forms, namely, as sulfurous acid (H_2SO_3), as potassium or sodium metabisulfite ($K_2S_2O_5$ or $Na_2S_2O_5$) and as the gas (SO_2).

The sulfurous acid and potassium or sodium metabisulfite were brought into contact with the grapes in the following ways: (1) by submerging the grapes in the containers in must which carried the desired amount of the preservative; (2) by immersing the grapes in the container for a definite period of time in a suitable water solution of the preservative; and (3) by transferring the desired amount of the preservative to the filled container of grapes with a small amount of water.

Immediately after the treatments by immersion and by the addition of the preservatives with a small amount of water, the kegs or barrels were rolled and turned end over end in order to bring the solution into contact with as many of the individual berries as possible. This facilitated uniformity of distribution in the container, since the sulfur dioxide is rapidly absorbed by the berries in contact with the solution.

The sulfur dioxide as a gas was brought into contact with the grapes directly. The gas was diluted with air in accordance with the concentration of SO_2 desired in the grapes and was then forced to flow rapidly through the filled containers of grapes for a definite period of time.

With less than 1000 mgs. per kilo. all lots treated by submerging the grapes in must to which the preservative was added in the form of sulfurous acid or potassium or sodium metabisulfite fermented in from 1 to 4 months. Series B, Cornichon, table 3, page 113, shows the results obtained in one lot treated with 650 mgs. SO_2 per kilo. in this manner. Treatments by the other methods, viz., (*a*) immersing the grapes in the container for a definite period of time in a suitable water solution of the preservative, (*b*) by transferring the desired amount of the preservative to the filled containers of grapes in just enough water to wet all the grapes in the container, (*c*) by exposing the grapes in the container to a definite concentration of SO_2 as a gas for a definite length of time, all gave equally good results, *whenever a uniform application was obtained.* It was difficult and uncertain, however, to obtain a uniform application by applying the preservative in just enough water to wet the grapes in the container, and this is therefore not a method to be recommended.

All lots of grapes treated by immersion or by gas as described, in which the amount of the preservative added was 600 mgs. per kilo. or more, retained practically all their original sugar content and showed no activity of micro-organisms for at least four months after treatment. Analyses of six of these lots in series B and C are given in tables 3 and 4.

Condition of the Grapes after Storage.—By suppressing the activity of micro-organisms the loss of sugar and the accumulation of alcohol were prevented. An effect of the SO_2 on the grapes, however, was to increase the permeability of the cells of all parts of the fruit and stems. The color was released from the skins, and the tannins and other substances from the seeds and stems. The berries showed varying amounts of "leakage" so that some free juice accumulated in the container. The appearance and condition of the fruit was decidedly not "fresh" and in no case would it be suitable for table fruit. For manufacturing purposes where the release of the color and tannins and other substances into the juice is not objectionable the grapes were nearly equal to fresh fruit. The color is temporarily bleached by the SO_2 but returns as soon as the preservative is removed.

SULFUR DIOXIDE IN THE PRESERVATION OF GRAPES IN OPEN CONTAINERS

Grapes have been "sulfured" by burning sulfur in the cars after loading for shipment to distant markets, at sporadic intervals for some eight or ten years. In the main these "sulfured" grapes have reached the markets in no better condition than the untreated shipments and in many instances their quality was impaired. In a few instances they have arrived on the distant markets in excellent condition. Adequate checks, however, have been lacking in all cases.

In the early work on the preservation of grapes in sealed containers, certain results obtained in tests on the rate of absorption of SO_2 by the grapes and the rate at which it was lost later when the grapes were exposed to laboratory conditions indicated that it might be possible to retard spoiling in open containers for a period of several days to several weeks. In the light of these results together with the fact that "sulfured" grapes have sometimes been shipped with partial success, tests were started in 1923 and continued with considerable elaboration in 1924, with a view of improving the method of application so that uniformly good results might be obtained.

RATE OF ABSORPTION BY GRAPES

SO_2 as a gas, when brought into contact with grapes, is readily absorbed by them. The rate of absorption of the SO_2 is influenced by the concentration of SO_2 in the atmosphere, the time of exposure, and by the temperature, maturity, and physical condition of the grapes.

Influence of Concentration and Time of Exposure.—Since the data on the relation of the concentration of the SO_2 and the time of exposure on the rate of absorption of the gas by the grapes were collected in the same tests they are presented together. Varying concentrations of SO_2 in air were forced over grapes in glass cylinders for given periods of time. The concentration of the SO_2 in the atmosphere was determined by absorption in iodine both before and after passing over the grapes. After the given time of exposure, representative samples of the grapes in the cylinders were removed and analyzed for SO_2. Representative results for several concentrations of SO_2 at the shorter periods of treatment are given in table 7.

The figures of table 7 show that at relatively low concentrations, the SO_2 is absorbed very rapidly from the atmosphere. The figures further indicate that the concentration of the gas as well as the time of treatment must be capable of careful control, and that the concentration of SO_2 must be uniform throughout the car. Only a small variation in the concentration of the SO_2 or the time of exposure may result either in too low a concentration of SO_2 in the grapes to suppress the activity of the micro-organisms or in a concentration sufficiently high to seriously injure the flavor and texture of the fruit.

TABLE 7

THE INFLUENCE OF THE CONCENTRATION OF SO_2 AND OF THE TIME OF EXPOSURE ON THE RATE AT WHICH THE GAS IS ABSORBED BY GRAPES

Variety	Per cent of SO_2 in the atmosphere	Mgs of SO_2 per kilo. absorbed			
		In 10 minutes	In 20 minutes	In 30 minutes	In 40 minutes
Valdepeñas	0.5				165
Muscat	2 to 3	60	85	114	
Tokay	2 to 3	58	64	93	
Muscat	4 to 6	99	251	656	
Tokay	4 to 6	70	171	547	

Influence of Temperature of the Grapes.—In these tests the range of temperatures occurring in practice was covered. The SO_2 gas was forced over the grapes in glass cylinders at the several temperatures. Uniformity of treatment was obtained by supplying the same volume of gas of the same concentration to all the cylinders at the same time. The volume of SO_2 supplied was very much greater than that absorbed by the grapes. The results obtained are shown in figure 2.

The graph of figure 2 shows that within the small range of temperatures usually met with in a car the matter of temperature of the grapes is of less importance than the concentration of the SO_2 or the duration of the treatments. There is, however, a direct relation between temperature and the rate of absorption; hence, where the temperature varies considerably as it may between cars at different seasons or in different localities, it must be taken into account in order to obtain uniform results.

Influence of Maturity of the Grapes.—This was determined by exposing grapes of various degrees of ripeness to a known concentration of SO_2 for a given period of time. The data shown in table 8 were obtained.

The figures of table 8 clearly show the necessity of having grapes of uniform ripeness where uniformity of treatment is desired. By varying the ripeness, as indicated by the Balling test, five degrees (from 18 to 13 in case of Muscat) the amount of SO_2 absorbed was more than tripled.

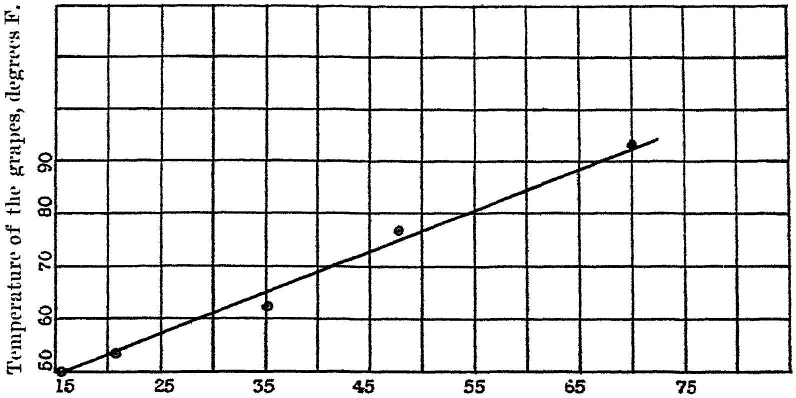

Fig. 2. The influence of the temperature of the grapes to the rate of SO_2 absorption.

TABLE 8

THE INFLUENCE OF MATURITY OF THE GRAPES TO THE RATE OF ABSORPTION OF SO_2

Variety	Mgs. of SO_2 absorbed per kilo. of grapes*		
	Ripe	Green	Very green
	(27° Bal.)	(18° Bal.)	(13° Bal.)
Muscat	43	77	262
	(23° Bal.)	(17° Bal.)	(10° Bal.)
Cipro Nero	110	129	218

* Treated for 7 minutes with a mixture of 4 per cent of SO_2 in air.

Influence of Condition (Soundness) of the Grapes.—Since the growth of micro-organisms and therefore the spoiling of grapes starts readily in injured berries, it is of particular interest to determine the rate and amounts of SO_2 absorbed by these berries as compared with sound grapes. To obtain data on the relation of injury to the rate at which the SO_2 is absorbed, a considerable number of Tokay and Muscat berries were carefully stemmed in such a way as to confine the extent of the injury to the removal of the stems. Stemmed berries and sound bunches of these varieties were then treated in identical fashion with regard to SO_2 concentration, air flow, and time of exposure. The results obtained are given in table 9.

The figures of table 9 show that stemmed berries absorbed SO_2 approximately twice as rapidly as sound berries. This more rapid intake of SO_2 by the injured fruit offers additional opportunity for getting the greatest possible retardation of spoiling by a minimum application of the gas. As spoiling usually starts in the injured berries, its arrest at this point through the absorption of larger amounts of SO_2 will largely remove the danger of spoiling in the sound grapes.

TABLE 9

THE INFLUENCE OF PHYSICAL CONDITION (SOUNDNESS) OF THE GRAPES ON THE RATE AT WHICH SO_2 IS ABSORBED

Variety	Condition of berries	Mgs. of SO_2 per kilo. absorbed from 2 to 3% in air		
		In 10 minutes	In 20 minutes	In 30 minutes
Muscat	Sound	60	85	114
	Stemmed	121	154	211
Tokay	Sound	58	64	93
	Stemmed	105	144	200

Application to Grapes in Open Containers.—In making these tests the conditions of the experiment were made as nearly as possible identical with those prevailing in an ordinary refrigerator car. Two air-tight rooms, each of approximately ¼ the size of a car, were used for the treatment and storage of the grapes. One of these rooms was equipped with an ice compartment for refrigeration so that the temperature could be maintained at 50° to 55° F. The other room was ventilated but not refrigerated. Both rooms were provided with floor racks.

The grapes were packed in Standard No. 1 (Los Angeles) lugs in the usual manner of "jumble pack." These lugs were not lidded and were stacked in the rooms in a manner similar to that used in the loading of a car, leaving spaces between the rows lengthwise of the room so as to permit the circulation of air horizontally and vertically.

The SO_2 was applied to the grapes in two ways: (1) By immersing the boxes filled with grapes in a solution of potassium metabisulfite of definite concentration for a definite period of time (see table 10, test no. 1); (2) as a gas.

The SO_2 for the gas treatments was produced by burning sulfur (1) in a specially constructed stove provided with a forced draft (see table 10, tests Nos. 2 and 3), and (2) in the closed rooms (see p. 128).

The special stove for the rapid generation of SO_2 by burning sulfur is shown in figure 3. It consisted essentially of an air-tight outside shell, constructed of thin sheet iron and concrete, into which was fitted an inlet at the bottom, an outlet at the top, and a tight fitting lid which could be opened so that the burner could be taken out and recharged. The burner consisted of an iron rack on which could be placed, in position one above the other, from one to five shallow pans filled with sulfur. This burner is similar to an arrangement recommended by the Gulf Sulphur Company for the rapid burning of sulfur. A forced draft was obtained by connecting the discharge of an electric vacuum cleaner fan with the inlet to the stove.

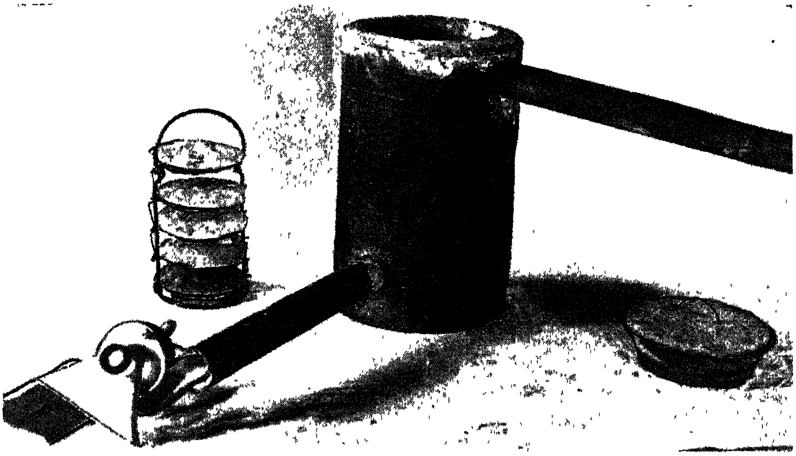

Fig. 3. Stove for the rapid burning of sulfur. Note the multiple pan burner and the fan for forced draft.

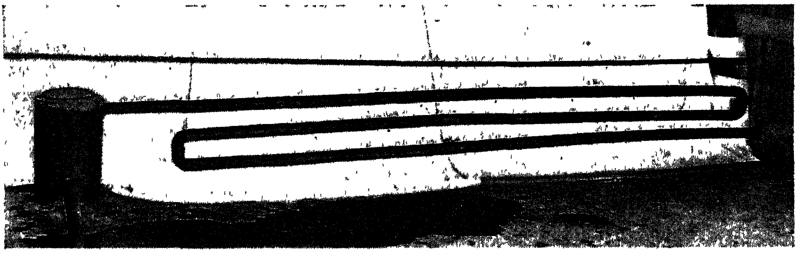

Fig. 4. Coil for cooling gas. Holes in the lower side of the pipe which supports the coil permitted the playing of cold water on all parts of the coil continuously.

Under this forced draft the sulfur burned vigorously, the heat from the lower pans vaporizing the sulfur in the upper pans. With this multiple pan burner, the concentration of SO_2 in the gas from the stove could be varied from 1 to 5 per cent by varying the number of pans used in the burner. The hot gas was cooled before being driven into the room where the grapes were placed, by carrying it through a long pipe over which a stream of cold water was kept running (see fig. 4). Some of the results obtained in these experiments are given in table 10.

TABLE 10

THE RELATION OF MANNER OF APPLICATION TO THE UNIFORMITY OF DISTRIBUTION OF SO_2 IN GRAPES WHEN STORED IN BULK AND THE EFFICIENCY WITH WHICH SO_2 RETARDS THE SPOILING OF GRAPES IN OPEN CONTAINERS.

Test No. and varieties	Average temperature of room	Position of the samples in the room			Mgs of SO_2 absorbed per kilo of grapes	Days elapsing before spoiling
		Stack	Row	Layer		
Test No. 1[1] with Sultanina.	76° F.	1	1	1	190	13 days
		1	3	6	78	10 days
		3	2	3	135	12 days
		5	1	6	74	10 days
		3	1	1 to 6	0 (check)	5 days
		2	2	1 to 6	48[3]	9 days
Test No. 2[2] with Muscat and Tokay.	74° F.	3	1	1 to 6	0 (check)	5 days
		5	3	6	80	15 days
		4	1	1	102	15 days
		4	1	4	99	15 days
		3	2	3	85	15 days
		2	1	2	90	15 days
		2	3	4	91	15 days
		1	1	6	91	15 days
		1	3	1	80	15 days
		5	1	1 to 6	0 (check)	5 days
Test No. 3[2] with Muscat and Tokay. (Grapes in poor condition, many broken berries.)	55° F.	3	1	1 to 6	0 (check)	7 days
		1	3	4	269	More than 18 days
		2	1	3	243	More than 18 days
		2	3	6	265	More than 18 days
		3	1	1	249	More than 18 days
		3	2	5	272	More than 18 days
		4	2	3	275	More than 18 days
		5	1	1	240	More than 18 days
		5	3	5	256	More than 18 days
		1	3	1 to 6	0 (check)	7-8 days

---

[1] Only one large pan used.

[2] Stove with multiple pan burner.

[3] Immersed for one minute in a 7% solution of potassium metabisulfite.

The outstanding facts brought out by the data of table 10 are: (1) that uniformity of distribution of SO_2 can be obtained when grapes are treated in air-tight rooms in which the arrangement of the boxes is similar to that of a loaded car, and (2) that grapes uniformly treated with the proper amount of SO_2 as a dry gas may be expected to keep at least twice as long as the untreated checks when held under the same conditions of storage. These statements are borne out by the figures obtained under tests nos. 2 and 3 as well as by those of the samples taken from and the returns obtained on a treated car which are reported on page 130.

Treatment by immersing the grapes in a water solution of potassium metabisulfite although resulting in a uniform distribution of SO_2 was not so effective in retarding spoiling as treatment with the gas, and the wet condition of the grapes after treatment detracted considerably from their appearance.

Uniformity of Distribution.—The fact that a uniform distribution of SO_2 in the room or car can be obtained when the SO_2 is applied in the proper manner is brought out in table 10, tests nos. 2 and 3 (also in the treated car reported in table 13).

The lack of uniform distribution in test no. 1 was the result of inadequate SO_2 supply rather than a fault of the method of application. In this test it required a considerable period of time (35 minutes) to burn the required amount of sulfur; hence, the displacement of the air in the room with the SO_2 air mixture was very slow and the grapes near the place of entry of the gas absorbed the greater amounts. In this case the sample from layer 1, row 1, and stack 1 with 190 mgs. SO_2 was directly in front of the gas inlet, while the sample from layer 6, row 1, and stack 5 with 74 mgs. SO_2 was from the part of the room farthest from the inlet.

In test no. 2, however, where the necessary sulfur was burned in less than ten minutes, the displacement of the air in the room with the SO_2 air mixture was very rapid and complete; thus all the grapes were exposed to an SO_2 air mixture of about the same concentration for the same length of time, which resulted in a nearly uniform absorption of gas by the grapes in all parts of the room. Here the sample, layer 1, row 3, stack 1, from directly in front of the inlet absorbed 80 mgs. SO_2, the same amount as that absorbed by the sample from layer 6, row 3, stack 5, from the farthest part of the room. Furthermore, the variations which did occur in the amounts of SO_2 absorbed by grapes from different parts of the room in tests nos. 2 and 3 were found to be the result of variation in the condition of the grapes such as Balling degree, injury, etc. (See pp. 121 and 122).

Retardation of Spoiling.—The data of table 10 indicate that only a relatively small amount of SO_2 need be absorbed by the grapes greatly to prolong their period of utility. In test no. 1, the treatment was not uniform and most of the grapes contained less than 100 mgs. of SO_2 per kilo., and yet they kept twice as long as the untreated check. The grapes of test no. 2, which received a very uniform treatment of from 80 to 100 mgs. of SO_2 per kilo., kept three times as long as the checks.

The question might arise as to why the checks spoiled so rapidly (5 days) in these tests. This is accounted for by the high temperatures of the storage rooms, which were 76° and 74° F. respectively.

In test no. 3 reported in table 10 the grapes were purposely injured through careless picking and through the transfer from field lugs to standard no. 1 lugs in order to see how this might influence the amount and uniformity of distribution of the SO_2 absorbed and the time elapsing before spoiling. A better idea of the condition of these grapes may be obtained by considering the fact that the untreated checks spoiled completely in seven days at 55° F. The SO_2 treatment given these grapes was identical with that of test no. 2 as regards concentration and duration. In test no. 3 the condition of the grapes, however, was such as to hasten the rate of SO_2 absorption (see table 9); hence, the amount absorbed was three times as much as that absorbed by the sound grapes of test no. 2. In spite of the poor condition at the time of treatment, these grapes kept nearly three times as long as the untreated checks.

Effect of Varying Amounts on Keeping Quality, Color, Texture, and Flavor.—In the application of SO_2 to table grapes or to any fruit of which the appearance greatly affects the sale value, the effect of the preservative on color, texture, and flavor must be considered as well as the retardation of spoiling.

To obtain information on the effect of varying amounts of SO_2 on the condition of the grapes and on the retardation of spoiling, grapes were carefully packed in large glass containers and then treated with varying amounts of the preservative. After the treatments, samples for analysis were removed for determining the exact amounts of SO_2 absorbed and the condition of the grapes of each container. The containers were then covered with cheese cloth to exclude vinegar flies and held at room temperature until spoiling occurred. The glass container permitted frequent observation of color and spoiling without disturbing the pack. The effects on flavor and texture were determined by examining and tasting samples removed from the container from time to time. The results of these tests are shown in table 11.

TABLE 11

THE EFFECT OF VARYING AMOUNTS OF SO_2 ON KEEPING QUALITY, APPEARANCE AND FLAVOR OF GRAPES

Variety	Mgs. of SO_2 per kilo. added to the grapes	Color and texture of treated grapes	Days elapsing before spoiling	Effect of the SO_2 on flavor
Black Prince	0 (check)		7 days	
	64	Normal	16 days	Flavor normal
Cipro Nero	0 (check)		6 days	
	46	Normal	13 days	Flavor normal
	70	Normal	20 days	Flavor normal
Malaga	0 (check)		8 days	
	35	Normal	14 days	Flavor normal
	92	Normal	16 days	Flavor normal
	201	Normal	20 days	Stale after 20 days. Trace of SO_2 in taste.
	451	Color lighter. Somewhat soft	25 days	SO_2 taste pronounced
Hunisa	0 (check)		8 days	
	19	Normal	11 days	Flavor normal
	38	Normal	14 days	Flavor normal
	185	Normal	20 days	Taste slightly injured
	454	Pale rose color Somewhat soft	25 days	SO_2 taste pronounced
Ohanez	0 (check)		10 days	
	13	Normal	16 days	Flavor normal
	43	Normal	22 days	Flavor normal
	125	Normal	30 days	Taste almost normal after 30 days
	256	Normal	30 days	Trace of SO_2 in taste

The data of table 11 indicate that about 50 mgs. of SO_2 per kilo. of grapes is sufficient to double the keeping period and that 100 mgs. does not injure the color, texture, or flavor of the varieties used in these tests. In view of these results it appears that the proper amount of SO_2 to apply to table grapes to delay spoiling in transit or storage is from 50 to 100 mgs. per kilo. of grapes.

Application by Burning Sulfur Inside the Closed Room.—Most of the "sulfuring" of loaded cars of grapes that has been attempted in the past has been done by burning sulfur inside the car after the doors have been closed. To test the efficiency of this method several experiments were set up in the tight rooms mentioned.

Burning the sulfur in open pans. In one set of these experiments an effort was made to generate the SO_2 by burning the sulfur in open pans inside the room. The grapes were packed and placed in the room as described on page 122. Three shallow pans, each containing from 1½ to 2 pounds of sulfur were placed side by side on the floor and ignited. The door and ventilators were closed. The results obtained in the most successful of this set of experiments are given in table 12.

TABLE 12

THE AMOUNT OF SO_2 ABSORBED BY GRAPES WHEN THE SULFUR WAS BURNED IN OPEN PANS IN A CLOSED ROOM*

Number of treatment	Pounds of sulfur burned	SO_2 concentration in grapes. Mgs. per kilo. after each successive treatment			SO_2 absorbed at each treatment (average)
		Minimum	Maximum	Average	
First	1.01	23	35	26	26
Second	1.8	....	....	60	34
Third	1.8	72	144	114	54

* The size of this room was about ½ the size of an ordinary refrigerator car.

It required three successive treatments to obtain over 72 mgs. of SO_2 per kilo. of grapes. The first application, in which a little over 1 pound of sulfur was burned, gave an average of only 26 mgs. per kilo. The second application, in which 1.8 pounds of sulfur were burned, gave only 34 mgs. per kilo. The successive treatments raised the temperature of the room considerably, so that the third treatment resulted in a somewhat greater increase in concentration (54 mgs. per kilo.).

When sulfur is burned inside an air-tight room, the oxygen content of the air is soon reduced below the concentration necessary to support combustion, and although a total of over 4½ pounds of sulfur was placed on the pans in the room not more than 1.8 pounds burned.

The data of this table also show that the distribution of SO_2 was not uniform, varying from 144 mgs. per kilo. near the burning sulfur to 72 mgs. per kilo. in another part of the room.

Results similar to these were obtained when the sulfur was placed in pans, one above the other, in the rack used in the burner for the stove discussed on page 123.

Burning the sulfur in a stove placed inside the room. By placing the stove described on page 123 inside the room, with the lid of the stove removed and the draft inlet connected to the air outside of the room and with the ventilators of the room open, more sulfur was burned, so that the required concentration of SO_2 in the grapes was obtained by one treatment. A forced draft was not used in these tests. However, the uniformity of distribution was no better and the heat produced by the burning of the sulfur raised the temperature of the grapes as much as 25° F. This excessive increase in temperature caused the treated grapes of these tests to spoil almost as soon as the untreated checks of other experiments. In the refrigerated rooms, it required several days to reduce the temperature to 60° after treatment in this manner.

Shipment of a Car-Load of Treated Grapes.—Through the cooperation of Mr. J. H. Wheeler of St. Helena, California, we were enabled to obtain samples for analysis as well as "returns" from a car of grapes treated with SO_2. Previous to the shipping of this car, Mr. Wheeler had treated a number of car-loads of wine grapes intended for short distance shipments and had obtained very encouraging results. The manner of application of SO_2 used by him in these treatments was practically identical with that used in most of the tests at Davis (described on pp. 123 and 124). This car, which was distinctly an experimental shipment to New York, contained a number of varieties in variable condition, as shown in table 13. After treatment, the car was kept closed for about 30 minutes. It was then entered and samples removed for analysis from the positions indicated in table 13.

The data presented in table 13 show that similar results, with regard to the quantity of SO_2 absorbed by the grapes and the uniformity of distribution, may be obtained in the treatment of car-loads as in the smaller air-tight rooms used for the experimental work at Davis. Furthermore, when account is taken of the condition of some of these grapes (Alicante Bouschet, Carignane, and Lenoir especially), which was such as to preclude shipment for more than a very short distance in the regular refrigerator car, the quality of the grapes when sold in New York, 18 days after treatment, and the price received, indicate that the treatment was of considerable value in lengthening the period of utility of these grapes.

TABLE 13

RESULTS OBTAINED FROM A CAR-LOAD OF GRAPES TREATED WITH SO_2 BY MR. J. H. WHEELER, ST. HELENA, CALIFORNIA

Variety	Condition when loaded	Position in car			Mgs. SO_2 absorbed per kilo. of grapes	Condition of grapes when sold in N. Y. 18 days after treatment*	Price received*
		Stack	Row	Layer			
Green Hungarian.	Very soft berries Many berries broken. (Balling 18.3)°	2	3	1	104.2	Very soft berries. Mostly good color, fair pack.	.90
		2	3	5	94.2		
		3	5	1	75.0		
		3	5	5	70.0		
		6	3	1	77.8		
		6	3	5	90.0		
		10	3	1	108.4		
		10	3	5	96.6		
		13	2	1	89.6		
		13	3	8	75.2		
		Middle of car					
		14	3	8	96.0		
Alicante Bouschet	Had been held on platform a week before loading. Berries soft, slight mold, color good.					Soft berries. Good color.	2.60
Carignane	Had been held on platform a week before loading Some berries raisined, slight mold, slightly off color (reddish).					Slightly raisined Slightly reddish color.	2.25
Lenoir	Had been held on platform a week before loading. Berries soft, slight mold, color good.					Good color. Very small bunches.	2.25
Petite Sirah	Had been held on platform a few days before loading. Berries soft, color good.					Very soft berries. Mostly good color.	1.90

* Report to J. H. Wheeler by the Earl Fruit Co.

SUMMARY

1. Of the commoner preservatives, sulfur dioxide, boric acid, formic acid, formaldehyde, benzoate of soda, and salicylic acid, sulfur dioxide alone is promising as a preservative for fresh grapes.

2. Sulfur dioxide in suitable concentrations prevents or retards the activity of all micro-organisms usually concerned in the spoiling of grapes.

3. A small quantity of sulfur dioxide decreases the rate of respiration in grapes.

4. With a suitable concentration of SO_2 uniformly applied to the grapes in sealed containers, it has been found possible to prevent the loss of sugar and the accumulation of alcohol almost indefinitely.

5. Uniformity of distribution of the SO_2 throughout the grapes in the sealed containers is absolutely essential for success.

6. The type and size of container, so long as it is air-tight and not easily corroded by acid, is of little or no consequence.

7. The SO_2 may be applied as a gas in air, or the grapes in the container may be immersed in a water solution of the gas or in a water solution of potassium or sodium metabisulfite for a definite length of time.

8. The treatment of grapes with SO_2 in sealed containers increases the permeability of the cells, so that the color, tannins, and other substances are released. This renders the fruit unsuitable for table use. For manufacturing purposes where the release of color and tannins into the juice is not objectionable, the grapes are almost equal to fresh fruit.

9. Through the uniform application of very small quantities of SO_2 to grapes in open containers, e.g. loaded cars, it has been found possible to double the period of time elapsing before spoiling with no injury to quality. Grapes treated in this way were in good condition for eating fresh.

10. The rate of absorption of SO_2 by grapes is influenced by (*a*) concentration of SO_2 in the atmosphere, (*b*) time of exposure, (*c*) temperature of the grapes, (*d*) maturity of the grapes, and (*e*) physical condition (soundness) of the grapes.

11. A uniform distribution of a suitable concentration of SO_2 without injury to quality may be obtained in grapes arranged as in loaded cars, when the following requirements are met: (*a*) a sufficient SO_2 supply and air flow to displace the air in the car with a uniform SO_2 air mixture within two or three minutes, (*b*) the SO_2 air mixture must be cool before being driven into the car, and (*c*) the equipment must be capable of maintaining this flow of SO_2 in air for from 10 to 30 minutes according to the concentration of the mixture used.

12. It was found that with a 2 or 3 per cent SO_2 concentration in the air, about twenty minutes was required to cause sound grapes to absorb 50 to 100 mgs. per kilo. With a 4 to 6 per cent SO_2 concentration in the air the time required was about ten minutes.

13. It was found that 50 mgs. of SO_2 per kilo. of grapes is sufficient approximately to double the keeping period and that 100 mgs. does not injure the color, texture or flavor of the grapes.

14. It was found impossible to treat grapes successfully by burning the sulfur inside the closed room or car.

15. The results obtained from an experimental car-lot shipment of grapes by Mr. J. H. Wheeler indicate that the results obtained in these experiments may be applied to commercial practice.

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GROUND WATER FLUCTUATIONS AT KEARNEY PARK, CALIFORNIA

BY

WALTER W. WEIR*

INTRODUCTION

Many of the problems which confront the engineer in irrigated regions would be much simplified if there were available reliable and detailed information concerning the fluctuation and movement of underground waters. Probably the two most important of these problems are the design of drainage works and the development of underground water supplies for irrigation.

The height to which the water table rises, the rate of rise and fall as it fluctuates at different seasons, the time at which maximum and minimum heights occur and the rate of yearly increase or decrease have an important bearing on the size, location, and depth of artificial drains and on the size, location, number and capacity of pumping plants, whether used for irrigation or drainage.

Unfortunately, the collection of data on ground water movements is seldom begun until its need becomes so urgent that studies can not be continued for sufficient time to make them entirely reliable. The studies carried on by the writer at Kearney Park near Fresno, California, are no exception to this rule. They are, however, of more than usual length and are in sufficient detail to be of considerable value for the purpose in mind, namely, the designing of a drainage system.

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* Assistant Professor of Soil Technology.

INSTALLATION

Although these observations were confined to Kearney Vineyard and interlying property at Kearney Park, the area covered (7000 acres) is sufficiently large and the soil conditions sufficiently like that found throughout some 100,000 acres in and about Fresno to make the results here obtained applicable to the larger area.

With minor exceptions, the depth to water was recorded weekly for eight out of the eleven years, 1912 to 1922 inclusive, in twenty-one regularly spaced test wells covering the area. In all, some 8000 observations were made. Figure 1 shows the relative location of the test wells and the general nature of the topography of the area.

The test wells consisted of auger holes ten feet in depth lined with 3-inch galvanized iron pipe. The top of the pipe projected about a foot above the surface. Each well was originally protected by being placed at the center of a triangle formed by three posts about which was wrapped three strands of barbed wire. This protection did not, however, in every case, withstand the attacks to which it was subjected by cattle and heavy farming machinery.

Being regularly spaced, these wells were subject to the natural variations which are likely to occur in an area of this size, such as variations in soils, topography, nature of tillage operations, irrigation requirements of different crops, distance from main supply canals and other local characteristics.

It will be noted, for instance, that wells 11, 12, 8, 9, and 3 are all on or adjacent to a low sandy ridge extending across the property, while wells 1 and 10 were influenced materially by seepage from the adjacent Fresno Sewer Farm.

PRESENTATION OF EXPERIMENTAL DATA

Although the factors mentioned unquestionably had considerable influence on the water table conditions surrounding any particular well and tend to emphasize the unreliability of individual observations, the fact that these variations exist should make the average results for the large series of wells of considerable general application. The height of the water as observed weekly was plotted for each of the twenty-one wells. Figure 2 gives the record sheets for wells 5, 10, 14, and 17, these being chosen as typical.

Because of the difficulty of keeping the wells in repair at all times, some of the early and late season readings do not give the full depth to water, the wells having become silted up. Note, however, was made of the actual depth at which they were found dry.

The actual elevations were obtained of the top of the well casing, to which all readings unless otherwise noted are referred, and of the surrounding ground surface. This information is shown on the record sheets.

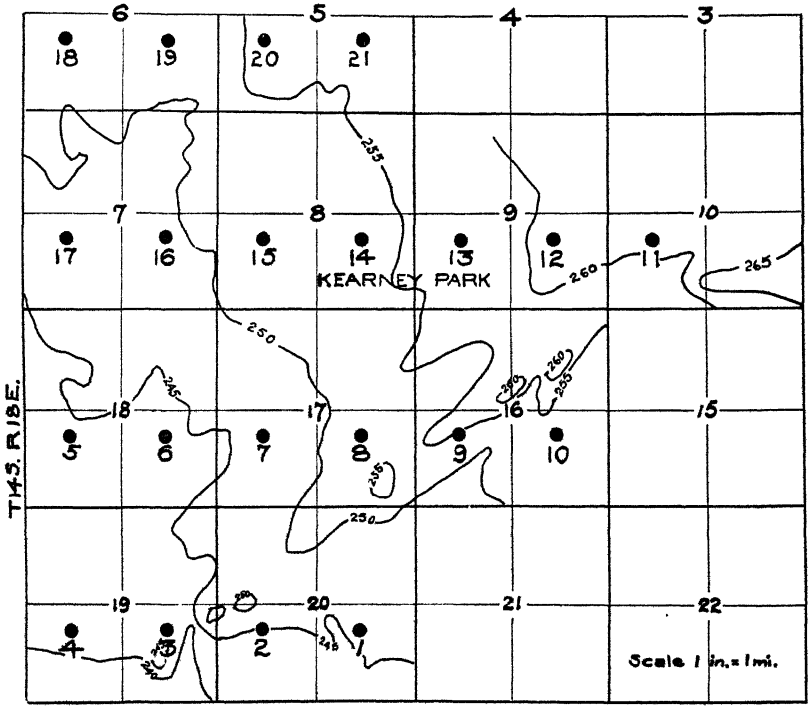

Fig. 1. Sketch showing location of test wells at Kearney Park.

In order to obtain a comprehensive view of the actual conditions uninfluenced by local happenings at any particular well, the curves of all the wells for any given year were superimposed and from them an average curve for the year was obtained. These average curves for each of the eight years are shown first in consecutive order in figure 3 and then superimposed in figure 4. It is from these two figures that the engineer can obtain the most data of real value.

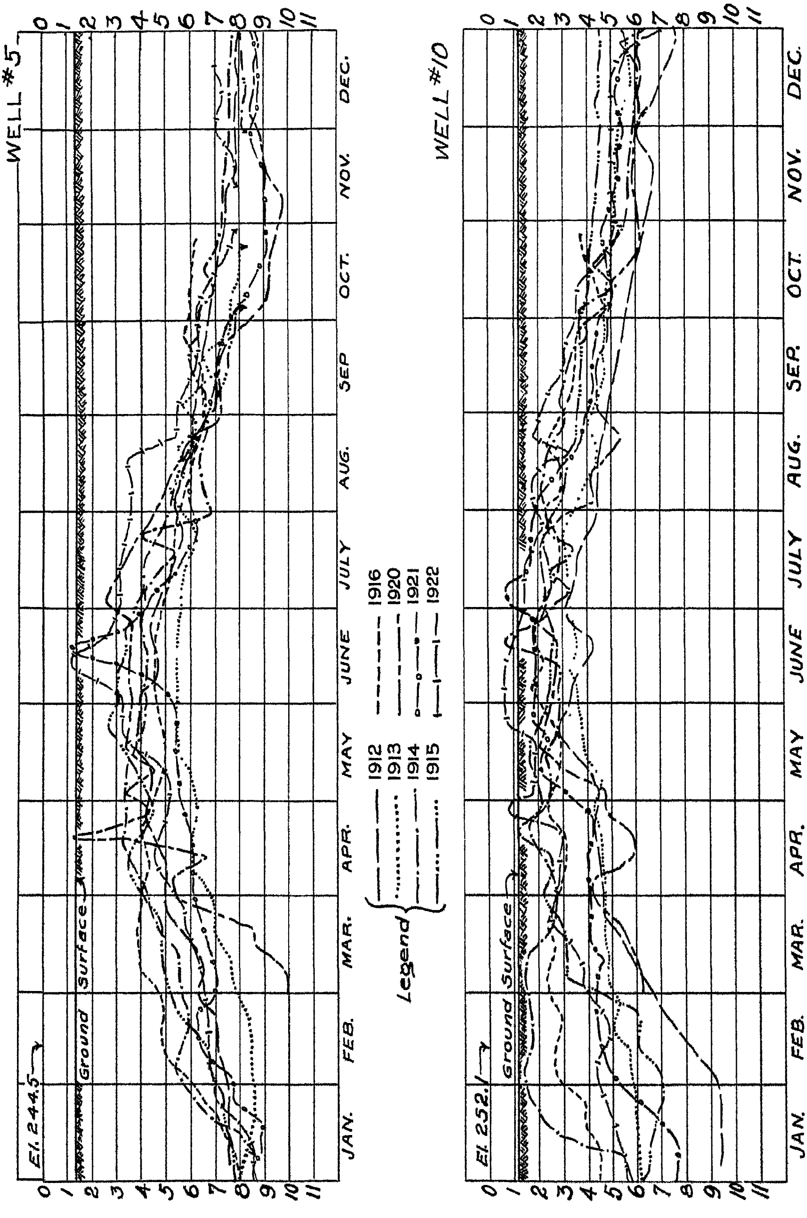

Fig. 2. Record sheets for four representative test wells, showing the position of the water table with reference to the ground surface.

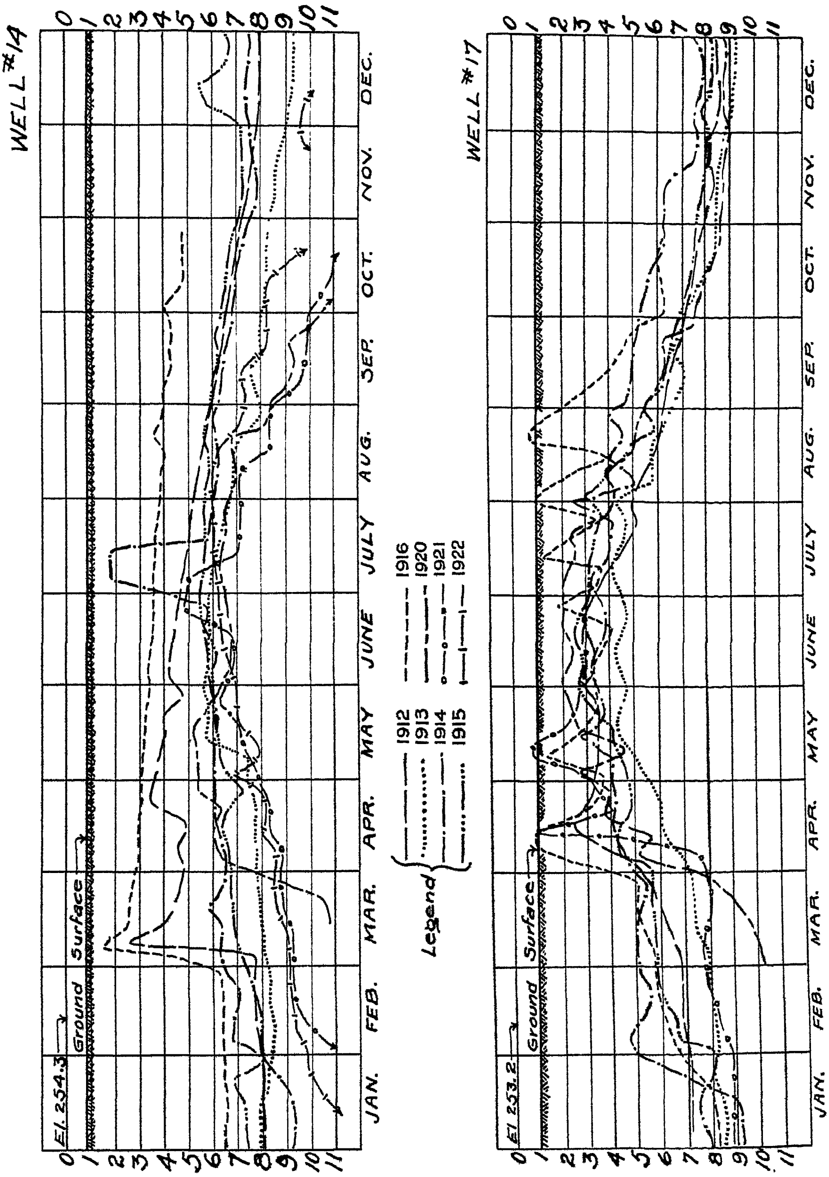

Fig. 2. (*Continued.*)

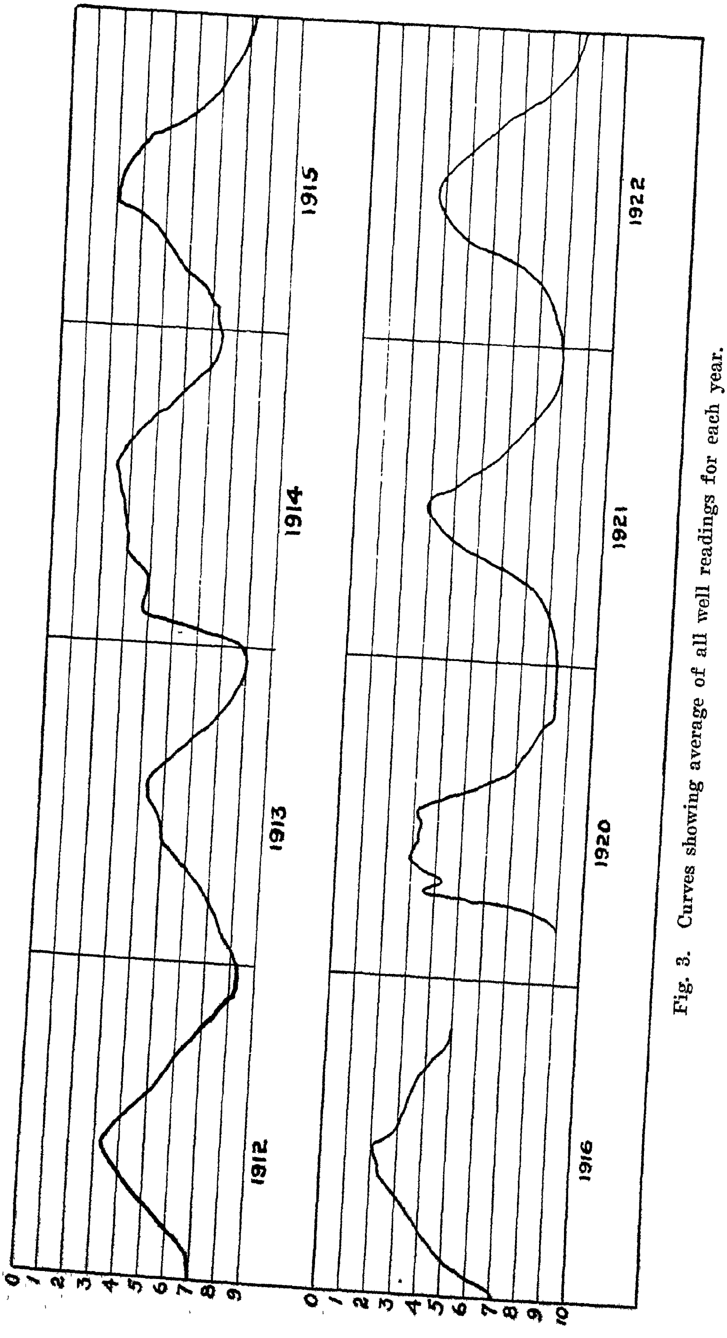

Fig. 3. Curves showing average of all well readings for each year.

Figure 5 was obtained by drawing an average curve through the eight yearly curves shown in figure 4. This curve shows more clearly the normal rate of rise over a considerable time, but is not so valuable in furnishing engineering data as those in figure 4. In the designing of drainage structures maximum rises and maximum supplies of water are controlling factors, while in the designing of irrigation structures the opposite conditions control.

In order to further bring out the fact that there is considerable variation between wells, table 1 is given, which shows for each well the approximate number of days in which the water stood at a given distance from the ground surface. This table was prepared from the data shown on the record sheets.

TABLE 1

TABLE GIVING FOR EACH WELL THE APPROXIMATE NUMBER OF DAYS WHEN THE WATER TABLE WAS WITHIN THREE AND WITHIN SIX FEET OF THE SURFACE

Depth	0′—3′								3′—6′							
Well No.	1912	1913	1914	1915	1916	1920	1921	1922	1912	1913	1914	1915	1916	1920	1921	1922
1	110	100	290	210	225	365	365	365	365	365	285	340	365	365	365	365
2	5	0	30	0	X	30	55	70	165	225	270	175	X	135	155	200
3	0	0	5	15	0	0	10	20	55	50	175	110	105	60	65	135
4	0	0	45	40	15	25	X	X	130	50	155	205	195	130	X	X
5	60	0	115	75	60	105	25	140	210	190	320	240	300	180	240	330
6	40	0	155	145	140	120	125	190	275	195	305	270	255	180	210	335
7	90	5	110	80	100	100	X	X	315	270	270	255	280	160	X	X
8	0	0	80	10	0	20	20	X	65	65	190	75	85	120	65	X
9	95	15	170	105	120	X	X	X	225	190	315	290	300	X	X	X
10	80	95	260	240	300	95	165	225	300	365	365	365	365	365	365	365
11	10	0	95	90	125	20	25	X	150	165	260	230	330	125	115	X
12	0	0	0	X	X	10	X	X	0	0	100	X	X	75	X	X
13	0	0	0	0	0	0	0	0	0	0	155	0	0	0	0	0
14	35	0	15	0	180	0	0	0	220	95	235	260	365	260	55	85
15	5	0	10	15	10	10	0	0	150	0	275	315	365	135	90	110
16	100	40	125	120	130	95	65	60	280	235	305	240	330	155	135	165
17	95	0	125	80	120	100	X	X	270	180	305	235	305	175	X	X
18	90	35	140	45	10	80	215	X	245	135	300	215	180	180	250	X
19	10	0	145	85	70	10	5	10	215	315	365	260	365	180	150	295
20	0	0	30	0	0	0	5	0	170	85	310	215	300	65	85	85
21	0	0	5	0	0	0	X	X	35	0	160	105	150	20	X	X

Note: X denotes records incomplete.

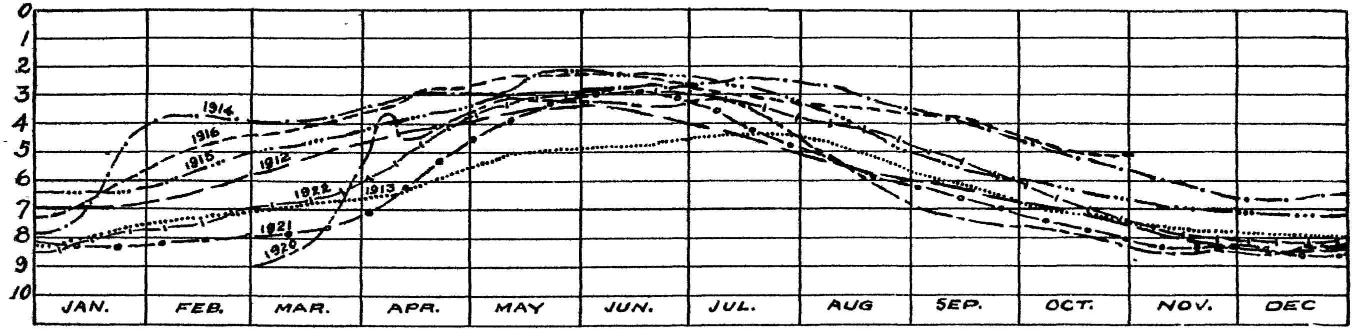

Fig. 4. Curves showing yearly fluctuations in water table, each curve being the average of weekly readings on twenty-one wells covering an area of 7,000 acres.

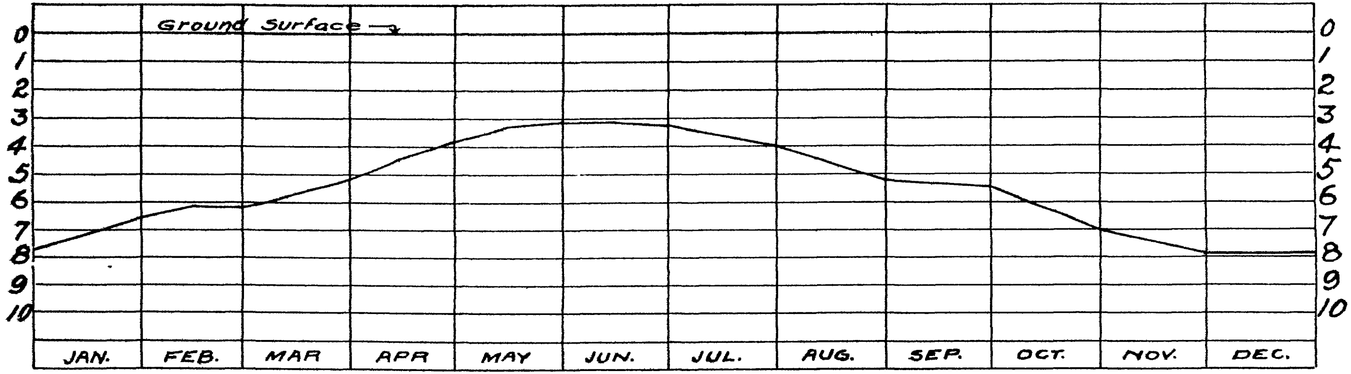

Fig. 5. Typical water table fluctuation curve showing the average of eight years' observations at Kearney Park.

OBSERVATIONS AND ANALYSIS OF DATA

From the data which have been secured, the following observations seem to stand out as most worthy of particular attention:

(1) Many of the wells show rather erratic fluctuations due to irrigation and when taken individually do not indicate actual conditions, except in the immediate vicinity of the particular well.

(2) The water table reaches a point nearest the surface during June. It is during this month that crops are growing in their most vigorous condition and abundant supplies of water are required to supplant that lost by transpiration. This water must be supplied by irrigation, which is frequently over-done, the excess going to raise the water table. During June, irrigation water is usually plentiful, but toward the end of the month and always in July, except in districts where storage water is available, there is a very rapid decrease in the supply. It is a common practice to apply excessive amounts of water in June in anticipation of a shortage in July. This fact is most strikingly illustrated by the conditions in the Turlock Irrigation District in 1923. Before 1923 no storage water was available and the usual practice of heavy irrigation in June was followed. In 1923, however, when late water was made available by storage in the Don Pedro reservoir, irrigation in June was more nearly in accordance with plant requirements, and as a consequence, the water table did not rise so high by several inches as it had done in previous years, although for the whole season about one-third more water was applied than had ever been used before.

(3) During most of the year, the water table is well within the ideal root zone of plants. During the mid-summer when root development should be the greatest and the feeding zone the most extensive, it is in reality most restricted because of the position of the water table.

(4) For the type of soil found in this region and with the shallow depth to the ground water, it is probable that water will rise to the surface by capillarity during the entire year, and during that part of the year when the temperatures are most favorable for high evaporation, the water table is nearest the surface. There must necessarily be a rapid accumulation of alkali at the surface under these conditions.

(5) During the season of high water table, the average distance of the water from the surface is not more than 2 feet, whereas during mid-winter the average is from 7 to 8 feet. The seasonal fluctuation is therefore between 5 and 6 feet.

(6) The most rapid rise in the water table occurs during March and April. This occurs within a short time after water is turned into the canals and the first irrigation of the season is applied. Seepage is probably excessive from the canals at this time because they have been dry during the winter months; evaporation and transpiration are at a minimum because the temperatures are relatively low and the plants small, and water is usually plentiful.

(7) As the season progresses, although the water table continues to rise until about the first of July, the rate of rise is much less than in the early spring. This may be accounted for by increased evaporation due to the rising temperatures, but it is more probably due to the greater use of water by the more abundant foliage of the crops.

(8) During June, there is very little fluctuation, but as soon as the water shortage begins in July the water table recedes. The recession is usually at a more uniform rate than the rise. By the first of December, the low point is reached and no particular change takes place during this month.

(9) The rate of rise in the water table after dry seasons is more rapid than in other years. The years 1913 and 1919 were both abnormally dry (this survey does not show the position of the water table in 1919), and it will be noticed on the following years, 1914 and 1920, the water table began to rise early and at a much more rapid rate than in other years. Also, that it remained at its high point for a much longer period. This is unquestionably due to the fact that water was applied in excessive amounts early in the season with the idea of overcoming the effects of the previous shortage and also in an attempt to save the crops from a possible shortage of water during the succeeding summer.

(10) There appears to be little or no tendency toward an annual increase in the height of the water table. In fact, there is an indication both from these data and from observations made elsewhere that 1914 is the year when the most undesirable conditions occurred. In order to give an exact account of the rise of the water table from depths of 60 or 80 feet, where it is said to have once stood, annual records for many years previous to 1912, when annual observations were first made, would be needed. However, in 1902, C. G. Elliott reports in U. S. Department of Agriculture Circular No. 50, drainage

conditions for this area that are almost as bad as those shown here, and before that date Dr. E. W. Hilgard in the California Experiment Station Report for 1886 sounds a warning on drainage for the country west of Fresno.

USE OF DATA

In designing drainage systems, provision must be made for removing the excess water in abnormally wet years, otherwise permanent crops such as orchards, vineyards and alfalfa may be injured. From the data at hand, it would then appear necessary to provide drainage of such capacity that rises in the water table similar to those in 1914 and 1920 could not occur, at least within the normal root zone.

The rate of rise and particularly the maximum rate is of as much importance in drainage design as the total annual rise. Since all of the fluctuations take place well above a safe drainage depth for irrigated regions, drainage to be adequate must be sufficient to preclude any rise whatever within the zone now affected.

From figure 5, it can be found that the average total effective rise for the eight years under observation was 4.35 feet, occurring within 135 days (January 1 to May 15). This is equivalent to an average rise of .032 foot per day. Assuming that for this particular soil the void spaces are 30 per cent of the volume, it would require 1.30 acre feet of water per acre to have caused this rise. This is, of course, in addition to that removed by deep percolation, evaporation and transpiration. From January 15 to January 27, 1914 (12 days), there was an average rise of 2.6 feet over the area or a rise at the rate of 0.216 foot per day. This is equal to the addition of 41.47 acre feet per square mile per day or 1 c.f.s. for each 30 acres under observation.

In 1920 from March 24 to April 7 (18 days), the rise was 4.3 feet or an average of 0.24 foot per day. This is the equivalent of 46.08 acre feet per square mile per day or 1 c.f.s. for each 27 acres. Assuming that 50 per cent of this could be removed from the soil by drainage, it would mean that drainage must be provided to remove 1 cubic foot per second for each 54 acres in order to prevent this rise.

For a large area a uniform system of drains of the capacity indicated would probably not be necessary, but the information given here, when taken in conjunction with that in table 1, will be of value in planning an adequate drainage system.

As an example of the practical use to which data of this nature may be put, the following drainage design may be mentioned. A system of drainage by pumping has been planned for the property upon which these data were collected. This system will consist of eleven pumping plants having an average capacity of 3.21 cubic feet per second which are to operate continuously from March 15 to December 1. During this period, it is estimated that a total of 17,000 acre feet of water will be discharged.

Although the 35 cubic feet per second, total capacity provided, is not sufficient to overcome a rise similar to that of April, 1920, the total discharge of 17,000 acre feet per year (2.43 acre feet per acre over 7000 acres or nearly twice the eight-year average) will lower the table so far below the surface that abnormal fluctuations should all occur at depths well below the danger point. In the case of tile or open ditch drainage, where the maximum drainage depth is usually but little below the required minimum, greater capacity would, of course, be necessary.

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THE EFFECT OF FEEDING *BACILLUS ACIDOPHILUS*, LACTOSE, DRY SKIM MILK, OR WHOLE MILK ON THE HYDROGEN ION CONCENTRATION OF THE CONTENTS OF THE CECA OF CHICKENS

J. R. BEACH

INTRODUCTION

The experiments, herein reported, were undertaken to determine in what manner, if any, the hydrogen ion concentration of the cecal contents of chicks would be influenced by feeding them with milk or certain milk products, and the relation of any changes found to occur to the control of coccidiosis.

Why milk feeding should be generally beneficial in intestinal infections of chicks is not made entirely clear by the published work of Rettger and his associates.[4, 5, 6, 7] The experiments of Beach and Corl[1] did not add any information regarding this point, although they proved the practical efficiency of milk in the control of avian coccidiosis. If the reasons for the value of milk feeding in the control of coccidiosis of chicks were known, it might be determined that some of the milk products which can be shipped long distances would be equally effective. In such a case a means of controlling coccidiosis would be available to poultrymen in localities for removed from dairying districts.

These studies have been confined to the ceca because coccidial infection of chicks is usually confined to these parts of the intestinal tract.

NOTE: The writer is indebted to Mr. L. A. van Rooyen for assistance in carrying out much of the detailed work recorded in this paper.

The chickens used in these experiments were confined in cages with grilled bottoms which allowed the droppings to fall onto paper-covered metal trays beneath. The openings in the bottom of the cage were too small to permit the birds to pick at the droppings that collected on the trays. The feed was given in metal cups attached to the grilled doors of the cages. Under these conditions, it was possible to maintain absolute control over all food and other material ingested by the birds.

After some preliminary work, the "spot plate" method was adopted for making the hydrogen ion concentration determinations.* Standard buffer solutions of known pH values at intervals of 0.2 were made according to the formulae of Clark.[3] Methyl red, brom-cresol purple, and brom-thymol blue were the indicators used. In making a pH determination, a small amount of material from the ceca was mixed with a few drops of neutral distilled water in a depression of the porcelain spot plate and one or two drops of indicator added. As a routine procedure, brom-cresol purple was first tried. If the pH was found to be out of the range of this indicator, additional tests were made using brom-thymol blue or methyl red. Comparisons were then made with mixtures of standard buffer solutions and indicator in adjoining depressions until an exact match in color was obtained and the pH value of the cecal material thereby determined.

The study of the flora of the intestines and ceca consisted simply in the microscopic examination of thin smears of the intestinal and cecal contents fixed in methyl alcohol and stained by Gram's method. Differential counts of the Gram-negative and Gram-positive organisms present were made. This count indicated any increase in the relative number of long, slender Gram-positive acidophilus-like rods that occurred.

THE EFFECT OF FEEDING CULTURES OF *Bacillus acidophilus*†

The birds used in this experiment had been confined in cages and fed an identical ration for more than thirty days before the experiment was started. It was thought, therefore, that the material in the intestinal tract of each should be quite uniform in character.

Before the culture feeding, as a means of determining the normal pH and flora of the intestinal tract of these birds, six of them were killed and examined. The results are given in table 1.

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* The writer is indebted to P. L. Hibbard for valuable advice and assistance in the selection of the method of making hydogen ion concentration determinations.

† The strain of *B. acidophilus* used in the preparation of the milk cultures for these experiments was obtained from Dr. L. F. Rettger.

But slight variation was found in either the pH or the flora of different birds. The pH of the duodenal contents ranged from 6.2 to 6.6, that of the cecal contents from 6.6 to 7.0. The proportion of Gram-positive rods to the total number of bacteria in the duodenal contents varied from 25 per cent to 41 per cent, and in the cecal contents from 32 per cent to 42 per cent.

TABLE 1

pH AND BACTERIAL COUNT OF DUODENAL AND CECAL CONTENTS OF NORMAL FOWLS

Fowl	Section of intestines	pH	Bacterial count
1	Duodenum	6.4	30% Gram+, few forms
	Ceca	7.0	42% Gram+; many forms
2	Duodenum	6.4	25% Gram+; few forms
	Ceca	6.8	32% Gram+; many forms
3	Duodenum	6.4	32% Gram+; few forms
	Ceca	6.8	40% Gram+; few forms
4	Duodenum	6.6	37% Gram+, few forms
	Ceca	6.8	34% Gram+; few forms
5	Duodenum	6.4	41% Gram+; many forms
	Ceca	7.0	41% Gram+; many forms
6	Duodenum	6.2	40% Gram+; many forms
	Ceca	6.6	33% Gram+; many forms

On July 31, 1923, *B. acidophilus* culture feeding was begun. One hundred c. c. of a 48-hour milk culture was given daily to each of twenty-six birds in individual cages. The milk was given in cups suspended on the cage doors. No other drink was allowed until the milk was consumed. The remainder of the diet consisted of whole wheat and cracked yellow corn, the maximum daily consumption of which was seventy-five grams. The milk, therefore, constituted more than half of the food. Starting on the third day and continuing at irregular intervals until the fifty-first day, the birds were killed for examination, one at a time, until twenty had been killed. In all cases, the birds were killed in the morning before the day's allotment of *B. acidophilus* culture had been given. Table 2 gives the detailed results.

No difference between the pH of the duodenal contents of these birds and that of the normal birds was found. The pH of the cecal contents in four birds was 5.6. In the others, it ranged between 6.0 and 7.0. The average pH, therefore, was slightly lower than in the birds that had not received the cultures. However, it was not demonstrated that the pH of the ceca was being progressively lowered since in the last three birds killed on the forty-sixth, forty-ninth and fifty-first days, the pH was 6.8, 6.0 and 6.6, respectively.

TABLE 2

pH AND BACTERIAL COUNT AFTER THE FEEDING OF *B. acidophilus* CULTURES

Fowl	Day killed	Section of intestines	pH	Bacterial count
1	3rd	Duodenum	6.4	96% Gram+; mostly acidophilus-like
		Ceca	7.0	52% Gram+; many acidophilus-like
2	5th	Duodenum	6.4	64% Gram+; few acidophilus-like
		Ceca	7.0	52% Gram+; very few acidophilus-like
3	8th	Duodenum	6.2	60% Gram+; very few acidophilus-like
		Ceca	6.8	64% Gram+; very few acidophilus-like
4	10th	Duodenum	6.4	52% Gram+; very few acidophilus-like
		Ceca	7.0	70% Gram+; many acidophilus-like
5	12th	Duodenum	6.2	62% Gram+; few acidophilus-like
		Ceca	6.6	57% Gram+; few acidophilus-like
6	15th	Duodenum	6.4	51% Gram+; many acidophilus-like
		Ceca	6.4	63% Gram+; many acidophilus-like
7	17th	Duodenum	6.4	100% Gram+; all acidophilus-like
		Ceca	6.8	46% Gram+; mostly acidophilus-like
8	19th	Duodenum	6.4	52% Gram+; 94% acidophilus-like
		Ceca	7.0	70% Gram+; 64% acidophilus-like
9	21st	Duodenum	6.2	50% Gram+; 66% acidophilus-like
		Ceca	5.6	68% Gram+; 99% acidophilus-like
10	23rd	Duodenum	6.4	76% Gram+; 95% acidophilus-like
		Ceca	5.6	65% Gram+; 92% acidophilus-like
11	29th	Duodenum	6.2	93% Gram+; 86% acidophilus-like
		Ceca	6.8	75% Gram+; 75% acidophilus-like
12	31st	Duodenum	6.2	85% Gram+; 83% acidophilus-like
		Ceca	6.6	71% Gram+; 71% acidophilus-like
13	35th	Duodenum	6.6	50% Gram+; 82% acidophilus-like
		Ceca	5.6	60% Gram+; 90% acidophilus-like
14	37th	Duodenum	6.4	70% Gram+; 62% acidophilus-like
		Ceca	6.8	90% Gram+; 88% acidophilus-like
15	39th	Duodenum	6.4	62% Gram+; 42% acidophilus-like
		Ceca	6.2	61% Gram+; 79% acidophilus-like
16	42nd	Duodenum	6.2	75% Gram+; 73% acidophilus-like
		Ceca	6.4	63% Gram+; 66% acidophilus-like
17	44th	Duodenum	6.4	77% Gram+; 61% acidophilus-like
		Ceca	5.6	61% Gram+; 95% acidophilus-like
18	46th	Duodenum	6.4	75% Gram+; 90% acidophilus-like
		Ceca	6.8	70% Gram+; 66% acidophilus-like
19	49th	Duodenum	6.4	80% Gram+; 72% acidophilus-like
		Ceca	6.0	50% Gram+; 86% acidophilus-like
20	51st	Duodenum	6.4	66% Gram+; 75% acidophilus-like
		Ceca	6.6	62% Gram+; 51% acidophilus-like

The differential bacterial count of smears of the duodenal and cecal contents showed an immediate and constant marked increase in the proportionate numbers of Gram-positive organisms, the major portion of which were of the acidophilus type. In the duodenum, the number ranged from 50 per cent to 100 per cent and in the cecum from 46 per cent to 90 per cent. This change in the flora, however, did not increase progressively with the continued feeding of the cultures; in fact, it was more marked in the bird that was killed on the third day than in the one killed on the fifty-first day.

This experiment has demonstrated, therefore, that feeding chickens milk cultures of *B. acidophilus* may cause organisms of the acidophilus type to predominate in the intestinal flora. It has not demonstrated, however, that the acidity of the intestinal contents will be thereby increased. These results are in agreement with those obtained by Rettger and Cheplin[8] in their experiments with rats and human subjects. It does not seem probable, however, that implantation of *B. acidophilus* is an important factor in coccidiosis control unless some other change, such as increase in acidity of the cecal contents, also results. Therefore, in the subsequent experiments, no study of the change in the intestinal flora was made, attention being paid only to changes in hydrogen ion concentration of cecal contents.

DETERMINATION OF CHANGES IN THE HYDROGEN ION CONCENTRATION OF CECAL CONTENTS BY EXAMINATION OF CECAL DROPPINGS

Browne[2] observed that only a small portion of the material passing through the intestines of a chicken enters the ceca. The coarser material passes directly from the small to the large intestine. A portion of the liquid and finely-divided particles enters the ceca, where it is retained for a considerable time. As a result, the cecal contents consist of a characteristic, homogenous, brown or chocolate colored, pultaceous mass, easily distinguishable from the contents of other portions of the intestines. Browne further observed that the ceca apparently do not continuously discharge into the large intestine but may completely empty themselves periodically after considerable material has accumulated in them. This material passes out in the droppings without becoming mixed with that from other portions of the intestines. Occasionally a dropping consisting entirely of such material from the ceca is passed.

The portion of the droppings coming from the ceca is easily distinguished by its characteristic color and consistency. This suggested the possibility of studying changes in the pH of cecal contents by the examination of the cecal droppings. Such a procedure, if successful, would make observations on the same bird possible as frequently as there were passages of cecal droppings. This would be more satisfactory than the single observation obtained by destroying the bird.

To obtain information on the accuracy of this method, several birds were kept under close observation and killed immediately after a passage of cecal droppings. The pH of the cecal droppings and that of the cecal contents of the same birds were found to be in close agreement, as shown in table 3.

TABLE 3

pH of Cecal Droppings and of the Contents of Different Parts of the Intestines

No. of bird	pH of cecal droppings	pH of different parts of intestines		
		Cecum	Duodenum	Middle of small intestines
19	6.4	6.4	6.2	6.2
22	6.2	6.0	6.2	6.6
13	6.2	6.2	6.2	6.2
12	6.6	6.6	5.6	6.8
14	6.0	6.2	6.2	5.6
17	6.6	6.6	6.4	6.8
4	7.0	6.8	6.0	7.0
5	6.4	6.6	5.8	7.0
18	6.2	6.2	5.8	7.0

This method of determination of the hydrogen ion concentration of the ceca was then applied to five birds remaining from the preceding experiment. Cecal dropping were collected each morning for eleven days. Table 4 gives the results of the pH determinations.

TABLE 4

pH of Cecal Droppings of Fowls Fed *B. acidophilus* Cultures

Fowl No.	pH of cecal droppings									
	Oct. 1	Oct. 3	Oct. 4	Oct. 5	Oct. 10	Oct. 11	Oct. 12	Oct. 13	Oct. 14	Oct. 17
22	7.0	6.0	6.4	6.4	6.4	6.4	7.0	6.8	6.8	6.8
23	—	—	6.4	6.8	6.6	6.6	6.4	—	6.8	6.8
24	—	—	6.4	6.8	6.8	7.0	6.6	6.6	—	7.0
25	6.8	—	—	6.6	6.8	—	6.8	6.8	7.0	—
26	6.8	6.8	6.8	6.8	6.8	7.0	7.0	6.8	7.0	7.0

"—"=No cecal droppings passed.

The limits of variation of the pH of the cecal droppings were 6.0 and 7.0. This is in conformity with the results of hydrogen ion determinations of the cecal contents of the birds in the preceding experiments. The determination of the pH of the cecal contents by this method, therefore, appeared to be accurate and was the procedure adopted for subsequent experiments. Because of the irregularity of the passages and the frequent admixture with urates encountered in the cloaca, it was not always possible to secure suitable samples of cecal droppings from each bird every day. In a large percentage of cases, however, two samples could be secured within twenty-four hours.

EFFECT OF FEEDING CULTURES OF *B. acidophilus* AND LACTOSE

Twenty-five cockerels were used in this experiment. From October 23, 1923, until November 6, each bird was given daily, as a drink, 100 c. c. of 48-hour milk cultures of *B. acidophilus*. This was placed before the birds between 9 o'clock and 10 o'clock each morning. Cultures alone were given for the first four days. During the next ten days, five grams of lactose was added to the milk for each bird. The lactose was then increased to 10 grams for each bird daily for the following nine days. The time required for the consumption of the 100 c. c. of cultures varied with the different birds from one to twenty-four hours. No other drink was allowed until the culture was consumed. Cecal droppings for pH determinations were collected on the morning that culture feeding was begun and on subsequent mornings at intervals of one to five days. In this and in all subsequent experiments, whenever droppings for pH determinations were collected, fresh paper

TABLE 5

pH OF CECAL DROPPINGS OF BIRDS FED DAILY 100 C.C. *B. acidophilus* CULTURES AND 5 GRAMS LACTOSE. LACTOSE INCREASED TO 10 GRAMS ON NOVEMBER 6

Fowl No.	Before feeding	Cultures alone		Cultures+5 grams lactose						
	Oct. 23	Oct. 24	Oct. 26	Oct. 29	Oct. 30	Oct. 31	Nov. 1	Nov. 5	Nov. 9	Nov. 14
1	5.6	6 0	6.0	5.3	4.8	6.6	6.8	5 6	5.4	5.2
2	6.0	6 0	6 8	5.4	5.4	6 4	5.8	5.8	5 4	6.6
3	6 6	6 4	6 4	5 4	6.4	5.8	6 4	5 8	5.6	6 4
4	6.2	6.2	6 2	6.5	—	5.8	5.4	5.6	5.0	6.4
5	6 0	6.2	5 4	—	5 4	5 6	5 6	5.4	—	—
6	6.4	6.8	6.2	6.4	6 4	5.8	6.6	6 8	6.6	4.8
7	6.2	6 0	5.4	6 4	6.6	5 2	6.0	5 0	—	—
8	6.2	6 8	6.2	—	—	—	—	5 8	5 6	5.0
9	5.4	6 8	6 6	5.2	6.0	5.8	5.4	6.6	6.4	5 0
10	6.2	—	6 6	4 8	—	5.6	5 6	5.2	5.0	6 8
11	6.4	6.2	5.4	5.0	5 6	6 8	5.2	—	5.0	6 6
12	6 2	6.2	6 2	4.8	5.8	6.4	5.0	5.0	4.8	6.2
13	6.6	6.4	5.6	4.8	5.8	6.4	5.4	5.4	5 4	5.4
14	6.2	—	—	6.2	6.2	—	—	5.6	4.6	6.2
15	6.4	6.2	5.4	5 6	5.4	6.0	5.8	5.8	6.2	6 0
16	6 6	6.8	6.6	5.8	5.8	6.4	5.4	5.0	6 2	6.0
17	6.2	6.4	6.4	5.6	5.8	6.0	6.4	6.2	5.6	6.8
18	6.2	7.0	5.4	7.0	5.8	7.0	6.8	6.8	—	—
19	6 4	6.4	6.8	5.6	5.4	6 6	5.4	6.2	5.2	4.8
20	6.4	6 4	6.4	6.8	6.8	6.2	5.4	6 0	4.4	—
21	6.6	6.8	5.4	6.6	6.8	—	6.8	6.8	5.2	6.4
22	6.4	—	6.4	6 4	4.8	6.6	5 8	5.4	5 0	6 6
23	6.4	6.8	6.4	4 4	4.8	6.6	5.4	5 4	4.8	—
24	6.8	7.0	6 4	4.8	5.6	6.6	6.6	—	5 4	5.4
25	6.2	—	5.4	5.6	5.8	—	—	—	—	—

"—"=Cecal droppings absent or mixed with other droppings.

was put on the trays. By this means it was known that all cecal droppings collected had been passed since the preceding collection of droppings. The results of the pH determinations appear in table 5.

As in preceding experiments, while the cultures alone were fed, the pH of the cecal droppings was not materially changed. After lactose was added, however, a marked lowering of the pH of the cecal droppings from all birds occurred. In the twenty-five birds the low points were as follows: one, 5.6; seven, 5.4; two, 5.2; six, 5.0; six, 4.8; one, 4.6, and two, 4.4.* The low pH, however, was not constant for the cecal droppings of any individual from day to day. Neither did this change become more marked with the increase of the daily allowance of lactose to 10 grams. The results of this experiment show, therefore, that when chickens are fed daily with cultures of *B. acidophilus* and lactose, acidity of the cecal contents may be produced, but they do not show that the acidity will remain constant during the feeding period.

VARIATION IN THE pH OF CECAL DROPPINGS DURING TWENTY-FOUR HOURS WHEN *B. acidophilus* CULTURES AND LACTOSE ARE FED

At this point, it occurred to us that the time of passage of cecal droppings with respect to the time of consumption of the *B. acidophilus* cultures and lactose might be a factor in the variation of the pH of the cecal droppings. Thus, for example, cecal droppings voided in the afternoon and evening, a few hours after the morning feeding of cultures, might be more acid than those voided during the night or morning, twelve or more hours after the cultures were consumed. Information on this point was furnished by a series of three experiments.

In the first experiment, twenty-four of the twenty-five cockerels of the preceding experiment were used. Cups containing 100 c. c. of *B. acidophilus* cultures and 10 grams of lactose were placed before the birds from 9.30 to 11.30 A.M. and then removed. Cecal droppings for pH determinations were collected before 10 o'clock in the morning and again before 5 o'clock in the afternoon. It was not possible to determine whether the droppings collected in the morning had been passed during the previous evening, during the night, or during the earlier morning hours. This was a possible source of error in the pH determinations for these droppings. It was definitely known, however, that all droppings collected in the afternoon had been passed since the morning feeding of the cultures and lactose. The pH determinations of the droppings are found in table 6.

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* In this paper when reference is made to an abnormal or high acidity, increased hydrogen ion concentration, low pH, etc., it indicates that the pH of the droppings is between 4.4 and 5.6. By normal acidity or hydrogen ion concentration is meant pH 6.0 to 7.4.

TABLE 6

pH OF CECAL DROPPINGS OF COCKERELS FED 100 C.C. OF *B. acidophilus* CULTURES AND 10 GRAMS OF LACTOSE FROM 9:00 TO 11:30 A.M.

Fowl No.	Nov. 15	Nov. 16		Nov. 20		Nov. 21		Nov. 22		Nov. 23		Nov.24
	P. M.	A. M.	P. M.	A. M.	P. M	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
1	—	5 4	5 0	5 8	—	5.0	—	5 4	—	5.6	5 0	6 2
2	—	5 4	—	—	—	5 2	—	6 2	—	6 4	—	6.4
3	—	5 4	5 2	—	5 2	6 2	—	6 2	—	6 4	5 2	6 2
4	—	6 4	—	5.2	5 2	6 4	—	6 8	—	6 8	5.4	6 4
6	—	6 0	5 0	—	4.8	6 8	5 0	6 8	—	7 0	5 2	6 8
8	—	—	6 0	5.2	—	5 0	5.0	6 0	5.2	5 6	5 0	—
9	—	6.2	5 2	—	5 0	6 2	—	6 4	—	6 2	5 0	6 4
10	5 0	—	5 0	—	5 2	6 8	5 2	6 4	5 0	6 8	5 2	6 8
11	—	—	—	5.8	5 2	5 4	—	6 6	—	7.0	5 4	7.0
12	5 4	—	—	—	5 0	5 0	5 0	5 6	5 4	6 4	—	6.4
13	—	6 2	5 0	6 2	5 4	5 2	—	6 2	5.2	6 0	5 0	6 6
14	—	—	5.0	—	5 2	5 4	—	6 2	5 2	5.4	—	—
15	5.6	5 8	—	—	5 4	6 8	—	6 4	—	6 0	5 2	6.8
16	—	5 8	—	—	5 2	6.6	5 2	6 2	5 4	6 2	5 4	6.6
17	5 4	5 2	5.2	5.0	—	5.2	5 2	5 2	5.2	5 4	5 2	6.4
19	5.2	6.0	5 4	—	5 2	6 0	5 0	5 4	5 0	5.4	5 0	6 4
20	—	—	—	—	—	—	—	—	—	—	—	—
21	—	6.6	—	5.4	—	6 4	—	6 2	—	6.4	5 2	6 8
22	5 6	—	5 2	—	5 2	6 2	5 4	—	5 2	6 2	5.0	6.2
23	5 0	—	5 0	—	—	5 0	4.8	5 0	—	—	—	—
24	—	—	—	—	5.0	5 0	5 0	—	—	6 4	—	—

"—" = Cecal droppings absent or mixed with other droppings.

A study of this table shows that with one exception (No. 8 on November 16, pH 6.0), the pH of all cecal droppings collected in the afternoon fell between 4.8 and 5.4. The droppings collected in the morning, however, showed the same pH variation as previously observed, slightly more than half being between 6.0 and 7.0 and the remainder between 5.0 and 6.0. It was noted that the afternoon passages of cecal droppings, the pH of which was low, were quite liquid or filled with gas bubbles. In this condition they quickly became mixed with other droppings on the trays which made it impossible to secure satisfactory samples in many instances.

Failures to secure both morning and afternoon samples of cecal droppings from the same bird, therefore, were frequent, but this was accomplished in forty-three instances. In twenty-six instances, the pH of the morning droppings was between 6.0 and 7.0 and that of the afternoon droppings from the same bird was from 0.8 to 1.8 lower or between 5.0 and 5.4. In the remaining seventeen instances, the difference in the pH was not marked, all of the morning determinations falling between 5.0 and 5.8 and the afternoon determinations between 4.8 and 5.4. In no case, however, was the pH of a morning dropping lower than that of an afternoon dropping from the same bird.

The procedure of feeding cultures of *B. acidophilus* and lactose and collecting cecal droppings twice daily for pH determinations was next applied to fifteen hens one year old. The pH determinations are recorded in table 7.

TABLE 7

PH OF CECAL DROPPINGS OF HENS GIVEN 100 C.C. OF *B. acidophilus* CULTURES, AND 10 GRAMS OF LACTOSE, FROM 9:00 TO 11:30 A.M.

Bird No.	Dec. 5		Dec. 6		Dec. 7		Dec. 10		Dec. 11	
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.
26	6.0	5.2	6.4	5.4	6.6	5.2	5.4	5.2	5.8	5.0
27	6.4	5.0	6.4	5.0	6.2	5.0	6.0	5.0	—	5.2
28	6.4	5.2	6.6	5.2	6.8	—	6.8	—	6.4	—
29	6.4	—	6.4	5.0	6.6	5.2	5.6	5.2	5.4	5.0
30	6.4	—	6.6	5.2	6.4	5.4	5.8	5.6	6.0	5.2
31	6.4	—	6.0	5.0	—	5.4	5.2	5.2	5.4	5.4
32	6.0	5.2	6.4	5.2	6.2	5.0	5.0	5.0	5.4	5.0
33	6.2	5.4	6.4	5.0	6.2	—	—	—	6.2	—
34	6.8	—	6.0	5.0	6.6	5.2	—	5.2	5.4	5.4
35	6.6	5.0	6.6	5.2	6.4	5.2	5.2	5.0	6.2	5.2
36	6.2	5.6	6.6	5.4	6.4	5.0	6.6	4.8	6.4	5.2
37	6.4	5.2	6.0	—	6.2	5.0	6.2	5.0	6.2	—
38	6.4	5.4	6.6	—	6.4	5.2	5.4	5.2	6.6	5.2
39	6.2	—	6.2	5.2	6.0	5.2	6.2	—	5.4	—
40	6.2	5.2	6.2	5.0	6.4	5.0	—	5.0	—	—

"—"=Cecal droppings absent or mixed with other droppings.

This table shows the results to correspond closely to those of the preceding experiment. The pH of the cecal droppings collected in the afternoon was uniformly between 5.0 and 5.4, while that of a majority of the samples of droppings collected in the morning was between 6.0 and 6.8.

The same fifteen yearling hens were used in the third experiment. Between 9 and 10 o'clock each morning, 50 c. c. of *B. acidophilus* milk culture and 5 grams of lactose were introduced into the crop of each bird with a pipette. This insured a uniform dose of cultures and lactose for each bird at a given time each day. Cecal droppings were collected and pH determinations were made twice daily as before. The pH determinations are given in table 8.

From this table it is seen that the pH of the cecal droppings, passed within six hours after the administration of 50 c. c. of *B. acid-*

ophilus cultures and 5 grams of lactose, was in every instance between 4.8 and 5.4. The pH of the cecal droppings passed from eight to twenty-four hours after the administration of the milk cultures and lactose, however, was in most instances above 6.0.

The results of this series of three experiments demonstrate that when milk cultures of *B. acidophilus* and lactose are fed to chickens, the acidity of the cecal contents, as indicated by hydrogen ion concentration determinations, increases within a few hours. This change, however, is of short duration as is shown by pH determinations made on the cecal droppings passed eight to twenty-fours hours after the *B. acidophilus* cultures and lactose had been fed.

TABLE 8

pH of Cecal Droppings of Hens Given 50 c.c. of *Bacillus accidophilus* Cultures and 5 Grams of Lactose with a Pipette

Bird No.	Dec. 12		Dec. 13		Dec. 14		Dec. 15		Dec. 18		Dec. 19
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
26	6 2	5 2	6 6	4 8	5 6	5 0	—	—	6.0	—	—
27	6 0	5 2	6.2	4.6	6 2	5 0	—	—	—	5 0	—
28	6.8	5 4	6 8	—	6 4	5 0	6.8	—	6 0	—	—
29	6 6	5.0	6 4	5.2	6 4	5.0	6 4	—	5 6	5.2	6.4
30	6 2	5 2	6 0	5 2	6 6	5 4	5 6	—	6 2	4 8	—
31	6.0	5 2	6 2	5.2	6 0	5 4	6 2	—	5 8	4.8	6.4
32	5.6	4 8	6.0	5 0	5.6	5 2	—	—	—	4 6	5.8
33	5.8	4 8	6 2	5 0	6 2	4 8	5 4	—	6.4	—	—
34	5.4	5 4	5.8	5 4	5 6	4 8	6 4	—	5.8	—	6.0
35	6.4	5.2	5 8	5.2	6.2	5 2	6.0	—	6.4	5 4	—
36	—	5.2	6 2	5.2	6.4	5.6	—	—	6.4	5 2	—
37	6.2	5 0	6.0	5 0	6 4	4.8	6 4	—	6.2	5.0	—
38	6.4	4 8	6.8	5.2	6.6	4.8	6 6	—	6.4	5.0	—
39	5.6	5.2	5.8	5 0	6 2	5.2	6.0	—	6 4	4 8	—
40	6 2	5.2	—	—	6.4	—	—	—	6.2	5.2	—

"—"=Cecal droppings absent or mixed with other droppings.

Effect of the Oral Administration of Lactose Alone

This experiment was designed to determine if by feeding chickens lactose alone the hydrogen ion concentration of the cecal contents would be changed to the same extent as when milk cultures of *B. acidophilus* were fed alone or combined with lactose. Ten yearling hens, divided into two groups of five each, were used. Each bird in one group received 5 grams of lactose each morning. In the other group, 5 grams of lactose was given to each bird morning and afternoon. The purpose of this was to determine whether by two feedings, the pH of the cecal contents would remain continuously low instead of for a few hours only after feeding, as occurred when *B. acidophilus* cultures and lactose were fed in the morning only.

Beginning on the day the first dose of lactose was given, cecal droppings for pH determinations were collected on five successive days. The pH determinations are given in table 9.

TABLE 9

pH OF CECAL DROPPINGS OF HENS GIVEN ONE OR TWO 5-GRAM DOSES OF LACTOSE DAILY

Bird No.	Jan. 28		Jan. 29		Jan. 30		Jan 31		Feb. 1		Feb. 2
	A. M.	P M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
One 5-gram feeding of lactose daily											
34	6 4	—	5 6	5 2	6 0	—	5 4	5 2	6 0	5 6	6 0
39	6 6	—	6 8	—	6 4	—	6 6	—	6 8	—	7.0
40	6 8	—	6 6	5 4	—	—	6 6	—	6 4	—	—
41	—	5 6	6 6	—	6.8	5 4	6 4	—	5 6	—	6 6
42	6 0	—	—	—	5 4	5 4	6.0	—	6 0	—	6 2
Two 5-gram feedings of lactose daily											
26	6 8	—	5 2	5 4	5 0	—	5 2	—	5 2	—	5 6
27	6 4	—	—	—	5 0	—	—	—	—	—	—
28	7.0	—	—	—	—	5.0	5 6	—	5 6	—	5 2
32	6.8	—	—	—	—	—	—	—	5 0	—	—
33	—	5.2	—	—	5.4	5 4	5 6	—	5.4	—	5.2

"—" = Cecal droppings absent or mixed with other droppings.

TABLE 10

A Reversal of the Groups in Table 9

Bird No.	Feb. 4		Feb. 5		Feb. 6		Feb. 7		Feb. 8		Feb. 9
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
One 5-gram feeding of lactose daily											
26	6 2	5 6	6 6	—	6 6	—	7 0	—	—	—	6 4
27	6 6	—	5 8	—	5 2	5 2	5.4	—	6 0	5 4	5 8
28	6.8	—	5 6	—	6 8	—	6.8	4 8	—	5.6	6 0
32	5 8	—	—	—	6 2	—	—	5 2	6.6	—	6.4
33	6 2	5.8	—	5.2	7.0	—	6.2	—	—	5.0	5.8
Two 5-gram feedings of lactose daily											
34	6 8	—	5 0	5 0	5 0	—	5.0	—	—	—	—
39	6.4	—	5 2	—	—	—	—	—	5 0	—	—
40	6 2	—	—	—	5 2	—	5 6	—	—	—	—
41	6 6	—	5 0	5.0	5 2	5 0	4.8	—	5.0	—	5 0
42	6 0	—	5.4	—	5 0	5.0	5 0	—	4.8	5.2	—

"—" = Cecal droppings absent or mixed with other droppings.

The watery and gaseous condition of the cecal droppings from these birds seemed even more pronounced than when birds were fed *B. acidophilus* cultures. As a result, failures to obtain suitable samples of cecal droppings were frequent.

The pH determinations of cecal droppings from the birds given lactose in the morning only were uniformly below 5.6 when collected in the afternoon, but, with few exceptions, were between 6.0 and 6.8 when collected in the morning. These results are in accordance with those obtained when *B. acidophilus* cultures, in addition to lactose, were fed. When lactose was given twice daily, however, with the exception of those collected on the morning of the first day before any lactose had been fed, the pH of both morning and afternoon cecal droppings ranged from 5.0 to 5.6.

This experiment was now repeated. The only change of procedure was a reversal of the two groups with respect to lactose administration. The birds in the group that formerly received one daily 5-gram dose of lactose now received two, and those in the group that formerly received two doses of lactose now received one. The purpose was to determine whether the uniform acidity of the cecal droppings, passed by the birds to which lactose was administered twice daily, was due to the administration of lactose and not to a peculiarity of the birds. The pH determinations are recorded in table 10.

These results are in accordance with those of the preceding trial. When the birds were given a 5-gram dose of lactose in the morning only, the pH determinations of the cecal droppings passed during the afternoon were between 5.0 and 5.6, while those of the cecal droppings passed during the night or in the morning ranged, with few exceptions, from 6.0 to 7.0. When the birds received 5-gram doses of lactose both morning and afternoon, however, the limits of variation of the pH of both the morning and afternoon collections of cecal droppings were determined to be 4.8 and 5.6.

The results of the three trials indicate that the hydrogen ion concentration of the cecal contents of chickens can be increased as readily and to an equal degree by the administration of 5 grams of lactose alone as by giving 5 grams or 10 grams of lactose plus 50 c. c. or 100 c. c. of milk cultures of *B. acidophilus*. The results also suggest that the hydrogen ion concentration of the cecal contents of a chicken that is given two 5-gram doses of lactose each day will be continuously greater than that of birds fed grain only.

EFFECT OF FEEDING MASH CONTAINING LACTOSE OR DRY SKIM-MILK

In the preceding experiment, it was found that a single oral administration of 5 grams of lactose would result in lowering the pH of the cecal contents. Within less than twenty-four hours, however, the cecal contents would again show a normal pH. When two 5-gram doses of lactose were given at an interval of about eight hours, the pH of the cecal contents appeared to remain continuously lowered. It seemed probable, therefore, that a low pH in the ceca could be more readily maintained if means were provided for a more or less continuous flow of lactose through the intestinal tract. It was thought that this could be effectively accomplished by mixing, with the food, lactose or some other dry milk product, such as dry skim-milk, which contains a large percentage of lactose. Dry skim-milk was thought to be particularly worthy of trial because of the suitability, as a food for poultry, of the ingredients it contains in addition to lactose. This composition, it was believed, would make its employment more practicable than that of lactose for field use in the control of coccidiosis in case it was found that lactose feeding was effective against the disease.

TABLE 11

pH OF CECAL DROPPINGS OF HENS FED DAILY 40 GRAMS OF MASH CONTAINING 5 PER CENT OF LACTOSE

Hen No.	Feb. 6		Feb. 7		Feb. 8		Feb. 9		Feb. 10		Feb. 11		Feb. 12		Feb. 13		Feb. 14	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
1	6 6	6 2	6 0	5.8	6.4	—	6.6	—	6.2	—	6.6	5 2	6 6	—	5 8	5.4	5 6	5.2
2	—	—	5 2	—	6.8	—	5.6	—	6 4	5 2	5.6	5.2	6.8	5.8	6 2	5 4	6.6	6.8
3	—	—	—	6.2	—	—	6.6	—	6 6	—	6.6	6 0	6.6	—	5.0	5 2	6.8	6.6
4	6.8	5.2	5.4	5.2	6 4	5.0	6.2	—	6 0	—	5 4	6 2	6 4	5.0	5.6	—	5.6	5.2
5	—	—	6.0	—	—	—	5.8	—	6 6	—	6 6	—	7.0	6 0	6.8	5.4	5.6	—

"—"=Cecal droppings absent or mixed with other droppings.

A series of four feeding trials was conducted therefore, to determine whether feeding mash containing lactose or dry skim-milk would cause the pH of the ceca to remain continuously lowered, and if so, what proportion of the total food consumption should be lactose or dry skim milk in order to bring about this change.

In the first trial, 40 grams of mash containing 5 per cent of lactose were fed daily to each of five yearling hens. Mash only was available to the birds from 10 a.m. to 4 p.m., but at other times mixed whole grain was also before them.

TABLE 12

PH OF CECAL DROPPINGS OF CHICKENS FED MASH CONTAINING 10 PER CENT LACTOSE (GROUP I) OR 20 PER CENT DRY SKIM MILK (GROUP II). FEEDING STARTED ON FEBRUARY 26

	Fowl No.	Feb. 26		Feb. 27		Feb. 28		Feb. 29		Mar. 1	Mar. 3		Mar. 4		Mar. 5		Mar. 6		Mar. 7		Mar. 8
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.
GROUP I	6	6.6	5.4	5.8	5.0	6.2	5.0	6.6	5.2	6.0	6.2	—	6.2	5.0	6.2	4.8	5.8	—	6.4	5.2	6.0
	26	—	5.2	6.8	5.0	6.8	6.0	6.2	5.8	6.8	6.4	5.0	6.4	6.2	6.4	5.2	6.8	5.0	6.6	5.0	6.8
	1	6.6	5.6	6.6	—	5.6	—	5.8	—	6.6	6.4	—	6.8	—	6.8	—	6.4	—	6.6	—	6.6
	2	5.6	5.4	6.4	—	5.4	—	5.4	—	5.4	5.4	—	5.0	—	6.0	—	—	5.2	6.4	—	6.6
GROUP II	4	6.8	5.4	6.6	4.8	6.8	4.8	5.6	5.6	6.6	6.8	—	5.8	6.4	—	—	5.4	—	7.0	5.4	6.8
	7	6.6	6.4	—	5.8	6.2	—	5.0	5.0	—	4.8	—	6.0	6.0	—	—	6.0	5.0	5.4	—	—
	8	5.0	5.6	5.6	—	5.0	—	5.6	5.6	5.4	6.2	—	6.2	6.0	—	—	6.0	—	6.2	—	—
	34	—	—	—	—	—	—	—	—	—	6.2	—	6.6	5.8	—	—	5.0	—	6.0	—	5.0

"—"=Cecal droppings absent or mixed with other droppings.

Cecal droppings for pH determinations were collected between 8:30 and 9:00 a.m. and between 4:00 and 4:30 p.m. for nine successive days. The results, as recorded in table 11, show that, while in many instances the pH of the cecal droppings was found to be between 5.0 and 5.6, this occurred in less than half of the pH determinations. It occurred with greater frequency in the afternoon droppings than in those collected in the morning. This shows that acidity of the ceca can be increased by feeding hens lactose mixed with mash, but indicated that the amount used in this case was too small.

In the second trial, two groups of four hens each were used. To Group I was fed mash containing 10 per cent of lactose, and to Group II mash containing 20 per cent of dry skim milk. The dry skim milk contained 50.6 per cent of lactose, which made the lactose content approximately the same in the mash for both groups. The amount consumed by each bird was determined by placing a weighed amount in the feed cups each morning and weighing out the unconsumed portion on the following morning. Cecal droppings for pH determination were collected twice daily in the first trial. The results are given in table 12.

It was found that in neither group was there a constant increase of acidity in the cecal contents. The pH of droppings collected in the afternoon was, with few exceptions, below 5.6, but in most instances the pH of the morning collection of night droppings was determined to be between 6.0 and 7.0.

The daily mash consumption by the individual birds varied from 45 to 120 grams. The daily lactose consumption by the individuals, therefore, varied from 4.5 to 12 grams in Group I and from 2.25 to 6 grams in Group II. The cecal droppings passed during the night following a day of heavy mash consumption frequently, but not uniformly, had a low pH value. It would appear, therefore, that to maintain the hydrogen ion concentration of cecal contents of chickens, constantly greater than that of birds fed entirely with grain, the food, irrespective of the amount consumed, must contain more than 10 per cent of lactose.

In the third trial with four hens, the percentage of lactose in the mash was increased to twenty. Cecal droppings for pH determinations were collected twice daily as before. The results of pH determinations are given in table 13.

By this table, it is seen that the pH values of all cecal droppings were between 4.8 and 5.6. These results, indicate, therefore, that an abnormal degree of acidity can be continuously maintained in the ceca of chickens when 20 per cent of the food is lactose.

TABLE 13

PH OF CECAL DROPPINGS AND FOOD CONSUMPTION OF HENS FED MASH CONTAINING 20 PER CENT LACTOSE. FEEDING STARTED ON MARCH 18, 1924

Fowl No.	March 18		March 19		March 20		March 21		March 22		Mar. 23
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
1	6.4	—	5.4	4.8	5.0	—	4.8	5.0	4.8	4.8	5.0
2	6.8	—	5.6	4.8	4.8	5.0	4.8	4.8	5.0	5.2	5.0
4	6.8	—	4.8	5.2	4.8	5.2	4.8	5.2	5.2	—	5.2
42	6.2	—	4.8	—	—	—	—	—	5.4	—	5.0

Grams of mash consumed

Fowl No.	March 18	March 19	March 20	March 21	March 22	Mar. 23
1	65	60	55	50	50	
2	100	85	100	100	90	
4	105	70	60	70	90	
42	110	70	70	65	30	

Lactose—equivalent in grams

Fowl No.	March 18	March 19	March 20	March 21	March 22	Mar. 23
1	13	12	11	10	10	
2	20	17	20	20	18	
4	21	14	12	14	18	
42	22	14	14	13	6	

"—" = Cecal droppings absent or mixed with other droppings.

In the last of the series of four feeding trials, mash containing 30 per cent of dry skim milk was fed to five hens. The pH values of the cecal droppings were determined twice daily and are recorded in table 14:

TABLE 14

PH OF CECAL DROPPINGS AND FOOD CONSUMPTION OF HENS FED MASH CONTAINING 30 PER CENT DRY SKIM MILK. FEEDING STARTED MARCH 24, 1924

Fowl No.	March 25		March 26		March 27		March 28		March 29		Mar. 30
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.
41	6.6	5.6	5.0	5.0	6.6	5.6	6.6	—	5.2	6.0	5.4
42	5.2	5.2	6.0	5.2	6.6	5.2	5.0	—	5.2	5.0	6.2
43	5.0	—	5.0	5.4	5.2	5.0	5.0	5.0	5.0	5.0	6.2
44	5.6	5.4	5.8	5.2	6.4	5.0	5.2	5.0	5.4	5.0	5.4
45	—	—	7.0	5.6	6.8	—	6.6	—	6.4	—	—

Grams of mash consumed

Fowl No.	March 25	March 26	March 27	March 28	March 29	Mar. 30
41	35	90	95	85	100	
42	60	45	75	90	120	
43	80	70	70	55	70	
44	70	95	95	95	100	
45	65	50	45	60	45	

"—" = Cecal droppings absent or mixed with other droppings.

In this case, all of the cecal droppings collected in the afternoon and many of the morning samples showed a pH of less than 5.6. The pH of morning samples, however, was frequently between 6.0 and 7.0. These results show that the amount of dry skim milk was too small. It seems reasonable to assume that had 40 per cent instead of 30 per cent dry skim milk been used, the results would have corresponded to those obtained with mash containing 20 per cent lactose.

THE RAPIDITY OF THE DEVELOPMENT AND THE DURATION OF THE INCREASE IN THE pH OF THE CECAL CONTENTS OF CHICKENS PRODUCED BY A SINGLE FEEDING OF MISCELLANEOUS MILK PRODUCTS.

These tests were designed to show the comparative effectiveness of sweet whole milk and the milk products that were used in the experiments discussed in preceding pages in lowering the pH values of the cecal contents of chickens. They were also expected to indicate the length of the time that acidity in the ceca produced by a single feeding would persist and thereby assist in determining the frequency with which feedings should be given in order to maintain the acidity in the ceca continuously. Individual birds were fed varying amounts of the different products and were killed at varying intervals afterward. The effect on the pH of the cecal contents was determined by comparing the pH determinations of the cecal droppings passed before feeding with those of the cecal contents after death. The number of birds used, the products fed, and the elapsed time between feeding and killing were as follows:

12 birds were fed from 50 c.c. to 75 c.c. of sweet whole milk and killed in from 1 to 2½ hours.

1 bird was fed 100 c.c. of milk cultures of *B. acidophilus* and killed in 2¼ hours.

9 birds were fed 50 c.c of milk cultures of *B. acidophilus* and 5 grams of lactose and killed in from 2 to 24 hours.

23 birds were fed 2 to 4 grams of lactose and killed in from 2 to 17 hours.

The results are recorded in table 15.

Summarizing the results as shown in the table, we find that:

No change had occurred in the ceca of the three birds killed in from 1 to 1¾ hours after they were fed from 50 to 75 c.c. of sweet whole milk.

Acidity had developed in the ceca of fifteen of nineteen birds killed in from 2 to 2½ hours after being fed 75 c.c. of sweet whole milk

TABLE 15

pH OF CONTENTS OF CECA OF CHICKENS KILLED AT VARYING INTERVALS AFTER ADMINISTRATION OF MISCELLANEOUS MILK PRODUCTS

	Age and sex of birds	Milk product	Amount	Time between feeding and killing	pH of cecal droppings before feeding	pH of cecal contents after death	
						Right	Left
32	Mature hen	Sweet whole	60 c c.	1 hr.	6.0	6.0	6.0
43	Cockerel	Sweet whole	75 c.c.	1½ hrs.	6.8	6.0	6.0
27	Mature hen	Sweet whole	50 c.c.	1¾ hrs.	7.0	6.4	6.4
34	Mature hen	Sweet whole	75 c.c.	2 hrs.	6.0	5.0	5.0
144	Cockerel	Sweet whole	75 c.c.	2 hrs.	6.8	6.6	6.4
145	Cockerel	Sweet whole	75 c.c.	2 hrs.	6.2	6.0	6.0
146	Cockerel	Sweet whole	75 c.c.	2 hrs.	6.6	5.4	5.6
147	Cockerel	Sweet whole	75 c c.	2 hrs.	6.0	5.0	5.0
148	Cockerel	Sweet whole	75 c.c.	2¼ hrs.	6.8	6.0	6.0
149	Cockerel	Sweet whole	75 c.c.	2¼ hrs.	6.0	5.2	5.4
410	Cockerel	Sweet whole	75 c.c.	2¼ hrs.	6.8	5.0	5.0
24	Mature hen	Sweet whole	75 c.c.	2½ hrs.	6.8	5.0	5.4
40	Mature hen	Acidophilus culture	100 c.c.	2¼ hrs.	6.6	5.0	5.4
20	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	2 hrs.	6.0	5.0	
16	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	2 hrs.	6.4	4.8	
4	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	4 hrs.	6.0	5.2	
6	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	4 hrs.	6.2	5.2	
3	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	6 hrs.	6.0	5.0	
9	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	6 hrs.	6.0	5.2	
11	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	8 hrs.	6.6	5.4	
10	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	24 hrs.	6.8	6.2	
15	Mature hen	Acidophilus culture +Lactose	50 c.c. 5 gms.	24 hrs.	5.8	6.6	
50	Cockerel	Lactose	4 gms.	2¼ hrs.	6.6	5.4	5.6
51	Cockerel	Lactose	4 gms.	2¼ hrs.	6.4	5.6	5.0
52	Cockerel	Lactose	4 gms.	2¼ hrs.	6.4	5.4	5.6
53	Cockerel	Lactose	4 gms.	2¼ hrs.	6.6	5.6	5.4
54	Cockerel	Lactose	4 gms.	2½ hrs.	6.0	5.0	5.4
55	Cockerel	Lactose	4 gms.	2½ hrs.	6.0	5.6	5.4
56	Cockerel	Lactose	4 gms.	2½ hrs.	6.6	5.8	6.0
101	Cockerel	Lactose	4 gms.	8½ hrs.	6.2	5.4	5.6
102	Cockerel	Lactose	4 gms.	8½ hrs.	6.4	5.2	5.4
103	Cockerel	Lactose	4 gms.	8½ hrs.	6.6	5.0	5.0
141	Cockerel	Lactose	4 gms.	8½ hrs.	6.8	5.4	5.8
142	Cockerel	Lactose	4 gms.	8½ hrs.	6.8	5.6	6.2
170	Cockerel	Lactose	4 gms.	8½ hrs.	6.4	5.2	5.4
64	Cockerel	Lactose	2 gms.	12 hrs.	6.0	5.4	5.6
66	Cockerel	Lactose	2 gms.	12 hrs.	6.0	6.4	6.0
67	Cockerel	Lactose	2 gms.	12 hrs.	6.6	6.6	7.0
70	Cockerel	Lactose	2 gms.	12 hrs.	6.4	6.4	6.2
41	Cockerel	Lactose	4 gms.	12 hrs.	6.2	5.2	5.2
42	Cockerel	Lactose	4 gms.	12 hrs.	6.6	5.0	5.8
44	Cockerel	Lactose	4 gms.	12 hrs.	6.6	5.0	5.6
22	Cockerel	Lactose	4 gms.	12 hrs.	6.8	6.4	5.2
45	Cockerel	Lactose	4 gms.	12 hrs.	6.6	6.6	6.2
1	Cockerel	Lactose	4 gms.	17 hrs.	6.8	5.6	6.8
2	Cockerel	Lactose	4 gms.	17 hrs.	6.2	6.6	6.4

100 c.c. of 48-hour milk cultures of *B. acidophilus,* 50 c.c. of milk cultures of *B. acidophilus* plus 5 grams of lactose, or 4 grams of lactose.

The acid condition was still present in two birds killed in six hours and in two killed in eight hours after being fed 50 c.c. of *B. acidophilus* cultures plus 5 grams of lactose; in five of six birds killed in 8½ hours after they were fed 4 grams of lactose; in one of four birds killed in 12 hours after being fed 2 grams of lactose; and in three of five birds killed in 12 hours after a feeding of 4 grams of lactose.

The pH values of the ceca of two birds killed 24 hours after the feeding of 50 c.c. of *B. acidophilus* cultures plus 5 grams of lactose, and of two others killed 17 hours after the feeding of 4 grams of lactose was that of normal cecal contents.

An important point not shown in the table is that cecal droppings of the birds killed in from eight to twenty-four hours after the feeding of a milk product showed a pH value lower than that found in the cecal droppings passed prior to the feeding. This is mentioned to show that when the pH values of the cecal contents of the birds that were killed in from 8½ to 24 hours after a feeding were found to be normal, it could be interpreted as a return to normal (6.0 to 7.4) from a lower point, not as a failure of the treatment to produce acidity in the ceca of the birds.

In most instances the pH values of both ceca of the same bird were in close agreement. Exceptions to this were found in birds Nos. 142, 22 and 1, in which the pH of the contents of the two ceca were 5.6 and 6.2, 6.4, and 5.2, and 5.6 and 6.8, respectively. This variation in the character of the contents of the two ceca of the same bird may be a source of occasional error in the interpretation of the pH value of cecal droppings as representing those of cecal contents of a bird.

The tests demonstrate, therefore, that a certain abnormal degree of acidity may be produced in the ceca of chickens within two hours after feeding suitable amounts of sweet whole milk, milk cultures of *B. acidophilus* or lactose. The acidity so produced may persist for from eight to twelve hours, but probably not for a longer period.

SUMMARY AND CONCLUSIONS

Feeding milk cultures of *B. acidophilus* to chickens resulted in the implantation of *B. acidophilus* in the ceca. In some instances, nearly 100 per cent of the bacteria present in smears of the cecal contents stained by Gram's method were of the acidophilus type. The implantation of *B. acidophilus* in the ceca of chickens, however, did not change the pH value of the cecal contents.

The part of the droppings of chickens originating in the ceca are voided separately and can be differentiated from the part of the droppings from other portions of the intestinal tract. It is possible, therefore, to study changes in the cecal contents of the same chicken that occur from day to day.

The pH of the cecal contents of chickens was changed from the normal range of 6.0 to 7.4 to a range of 4.4 to 5.6 by feeding sufficient amounts of whole sweet milk, milk cultures of *B. acidophilus,* milk cultures of *B. acidophilus* plus lactose, lactose alone, or dry skim milk.

Since lactose is the only ingredient common to all of the milk products used, the change in hydrogen ion concentration of the cecal contents produced by feeding milk or a milk product would appear to be due to the lactose it contains.

The change in the hydrogen ion concentration of cecal contents from a single feeding of a milk product occurred within two to two and one-half hours after the feeding and returned to normal within eight to twenty-four hours after the feeding. The rapidity of development and the short duration of the change in hydrogen ion concentration indicates that it is not a result of modification of the flora of the intestinal tract.

An abnormal degree of acidity in the ceca was constantly maintained by the individual administration to chickens of one or two grams of lactose twice each day at an interval of about eight hours, or by the continuous feeding of mash mixtures containing 20 per cent of lactose. The feeding of mash containing 40 per cent dry skim milk would also provide approximately 20 per cent of lactose in the mash and should, therefore, accomplish the same result.

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THE INFLUENCE OF FEEDING LACTOSE OR DRY SKIM MILK ON ARTIFICIAL INFECTION OF CHICKS WITH *EIMERIA AVIUM*

J. R. BEACH AND D. E. DAVIS

INTRODUCTION

The experiments reported in this paper consist of a series of five trials in which it was attempted to combat artificially-produced coccidial infection in chicks by feeding them with sufficient lactose or dry skim milk to change the hydrogen ion concentration of the ceca from the normal range of 6.0–7.4 to a range of 4.4–5.6. It was thought that, by this means, an environment unfavorable or destructive to the tissue-invading stages of the parasite, viz., the sporozoites and merozoites, might be created.

The first three trials were carried out under laboratory conditions, the chicks being confined in cages with grilled bottoms and fed in cups suspended on the cage doors. In the last two trials, the chicks were reared in brooder pens under normal field conditions, except that no outside runs were provided.

After the feeding of lactose or dry skim milk was begun, the chicks were inoculated by introducing into their crops with a pipette a large number of sporulated oöcysts of *Eimeria avium.* A control group of chicks that was fed neither lactose nor dry skim milk was included in each trial. An estimate of the number of cysts administered to each chick was obtained by making a direct microscopic count of the cysts in $1/100$ c.c. of the inoculum. Material for inoculation was provided by cultures of the cecal contents of chicks affected with coccidiosis prepared as follows: A thin layer of cecal contents containing large numbers of oöcysts was spread over the surface of salt solution agar plates.[1] Salt solution to keep the surface of the plates moist was added as required. The cultures were incubated at room temperature until microscopic examination showed that sporulation of the oöcysts had occurred.

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[1] The writers are indebted to H. W. Graybill for suggesting the use of and furnishing the formula for the "salt solution agar." The formula is as follows: Agar, 20 gms.; sodium chloride, 5 gms.; distilled water, 1000 c.c. The agar is cut up, tied in a gauze bag and washed for two hours in running water before the medium is made up.

TABLE 1

pH OF CECAL DROPPINGS AND EFFECT OF INOCULATION WITH SPORULATED OÖCYSTS IN FIRST TRIAL

	Fowl No.	Mar. 15		Mar. 16		Mar. 17		Mar. 18		Mar. 19		Mar. 20		Mar. 21		Mar. 22		Mar. 23		Mar. 24		Mar. 25	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M	P.M.	A M.	P.M.
Group I (Lactose)	51	5.4	5 6	—	—	5 4	—	X	—	—	5.6	—	X	—	5 6	—	—	—	—	X	5 0	5 2	—
	52	—	—	5.6	—	X	—	5 6	5 6	—	—	5 6	—	B	—	5 6	—	—	—	—	—	—	—
	53	5 6	4.8	—	—	X	—	5.4	5 2	—	5 0	5.0	5 0	—	—	5 0	—	—	—	5 2	5 2	—	—
	54	—	4.8	—	—	X	—	X	—	—	—	5 6	5 4	B	—	B	—	B	—	—	—	—	—
	55	5.4	5 2	5 4	—	5 0	—	5 0	5 4	5 6	—	5 2	5 2	5 4	5 4	5 2	—	5.4	—	—	5 4	5 4	5 2
Group II (Controls)	56	6 0	6.0	5.6	—	5.8	—	5.6	5.6	5.8	—	—	—	B	B	B S	S	S	S	D			
	57	6.2	5.8	6 2	—	6 4	—	6 2	—	6 2	6 0	B	B S	D									
	58	6.2	—	6.0	—	6 0	—	6 0	—	5 4	—	B	B S	B S	D								
	59	6.8	—	6 6	—	6 4	—	6 6	—	6 0	5 4	B	B S	D									
	60	6.4	—	—	—	6.0	—	—	—	—	—	—	—	B	B	B	—	—	—	—	—	—	—

S=Bird visibly sick.
B=Cecal droppings contain blood.
D=Died from coccidiosis.
X=Cecal droppings watery and mixed with other portion of droppings.
—=No cecal droppings.

FIRST TRIAL

Ten chicks, four weeks old, were divided into two groups of five. They had been reared in an environment thought to be free from *Eimeria avium.* From March 14, 1924, each bird in Group I was given 1 gram of lactose twice daily at 9 a. m. and 4:30 p. m. Group II, the control, received no lactose. On March 15, approximately 45,000 sporulated oöcysts were introduced into the crop of each bird in both groups. Cecal droppings for pH determinations were collected twice each day. The pH determinations of the cecal droppings and the effect of the inoculation on the chicks are recorded in table 1.

The pH determinations of the cecal droppings showed a constant higher degree of acidity in the ceca of the birds of the lactose group than in the controls.

Blood appeared in the droppings of two birds (nos. 52 and 54) of the lactose group on the sixth day. No. 52 passed blood for one day only, but No. 54 continued to do so for three days. Merozoites were present in the bloody droppings from these birds. None of the birds in this group were otherwise visibly affected. Oöcysts were found in the droppings of all birds after the sixth day.

Three of the five controls were passing bloody droppings on the fifth day. All passed blood and three died from coccidiosis on the sixth day. The fourth death from this cause occurred on the ninth day. The one remaining bird ceased passing blood after three days and exhibited no further symptoms.

All the birds were killed for autopsy on the eleventh day. The ceca of four of those in the lactose group appeared to be normal. One cecum of No. 52, was filled with a caseous core.

Both ceca of the one survivor of the control group were filled with a bloody, caseous core.

The results indicate that the lactose feeding was of marked benefit in combatting artificial infection with sporulated oöcysts of *Eimeria avium.* Two of the birds in the lactose group passed bloody droppings in which merozoites were present, and oöcysts occurred in the cecal droppings of all birds. This is evidence that at least a part of the sporozoites released from the sporocysts were unharmed and invaded the cells of the cecal mucosa where both the sexual and asexual cycles of development were completed. It is possible that the dose of oöcysts was too large to be entirely overcome or that the increased acidity in the ceca was more destructive to the merozoites than to the sporozoites.

TABLE 2

pH OF CECAL DROPPINGS AND EFFECT OF INOCULATION WITH SPORULATED OÖCYSTS IN THE SECOND TRIAL

	Bird No.	Mar. 29		Mar. 30		Mar. 31		Apr. 1		Apr. 2		Apr. 3		Apr 4		Apr. 5		Apr. 6		Apr. 7		Apr. 8
		A.M	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P M.	A.M	P.M.	A.M.	P.M.	A.M.
Group I (Lactose)	61	5.6	5.2	5.4	X	5.4	X	5.2	5.4	5.2	5.0	5.6	5.2	5.4	5.4	5.6	—	B	B	B	—	X
	62	5.4	5.2	5.2	X	5.0	5.0	5.4	—	5.0	5.4	5.4	5.4	5.2	5.4	5.0	X	B	—	B	—	X
	63	5.2	5.4	5.2	X	5.2	5.2	5.2	5.6	5.6	5.2	5.4	5.4	5.4	5.6	5.4	X	B	B	B	—	X
	64	5.0	5.0	5.4	X	5.4	X	X	X	5.4	—	5.2	—	5.0	X	—	X	D	—			
	65	5.2	5.2	5.0	X	5.2	X	5.4	5.0	5.2	5.2	—	5.0	5.2	5.2	X	—	B	B	B	—	X
Group II (Controls)	66	6.4	—	6.6	—	5.4	—	6.2	—	6.6	—	6.4	6.0	6.0	B	B S	—	D				
	67	6.8	—	6.4	—	6.6	—	6.0	—	6.6	—	6.8	6.2	6.0	—	B S	—	D				
	68	6.8	—	6.2	—	5.8	—	6.6	6.4	—	—	6.0	—	—	B	B	—	B	B S	S		S**
	69	6.6	—	—	—	6.0	5.6	6.6	—	6.0	—	6.0	—	6.4	B	B S	—	B S	D			
	70	6.2	6.2	6.2	—	6.0	—	6.4	6.4	6.6	—	6.4	—	5.6	—	B S	—	B S	B S	S		S D*

S=Bird visibly sick.
B=Cecal droppings contain blood.
D=Died from coccidiosis.
X=Cecal droppings watery and mixed with other droppings.
—=No cecal droppings.
*=No. 70 died on Apr. 12.
**=No. 68 recovered.

SECOND TRIAL

Ten chicks, six weeks old, were divided into two groups of five. The method of procedure was the same as in the first trial. The feeding of lactose to the birds in Group I was started on March 28, 1924. Three days later, approximately 40,000 sporulated oöcysts were introduced into the crop of each bird of both groups. The pH determinations of the cecal droppings and observations on the effect of the inoculation are given in table 2.

As in preceding experiments, an abnormal degree of acidity in the ceca was produced by the feeding of lactose.

One bird of the lactose group began voiding bloody droppings on the fifth day and three others on the sixth day. Both merozoites and oöcysts were found in the droppings. The appearance of the droppings had become normal on the eighth day and these birds exhibited no further symptoms. The fifth bird of Group I was found dead from coccidiosis on the morning of the sixth day. It had not previously appeared sick nor passed blood with the droppings.

Three of the control group were voiding bloody droppings on the fourth day. On the fifth day all showed marked droopiness and were passing blood. Four died from coccidiosis, three on the sixth day and one on the twelfth day. The remaining bird was visibly sick for several days but finally recovered in so far as the manifestation of symptoms was concerned.

All survivors were killed for autopsy on the fifteenth day after inoculation. The ceca and other organs of the four birds of the lactose group were normal in appearance. Microscopic examination of the cecal contents for coccidial cysts was negative.

The ceca of the one survivor of the control group were found to be entirely filled with solid, bloody, caseous cores which contained numerous oöcysts.

These results closely parallel those of the preceding trial. It was again demonstrated that feeding lactose did not prevent the sporozoites from invading the epithelial lining of the ceca where the cycles of development of the parasites were completed. In four of the five birds, however, further development of disease was arrested. The death of the one bird might have resulted entirely from the tissue damage caused by the invasion of the sporozoites and completion of their developmental cycles. This would appear to be additional evidence that the acidity produced in the ceca by feeding lactose to chickens is more destructive to the merozoite than to the sporozoite forms of *Eimeria avium*.

TABLE 3

pH OF CECAL DROPPINGS OF BIRDS IN THE THIRD TRIAL

	Cage No.	Mash fed	Number of birds	May 21		May 22		May 23		May 24		May 25		May 26		May 27		May 28		May 29	
				A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A M.	P.M.	A.M.	P.M.	A.M.	P M.	A M.	P.M.	A.M.	P.M.
Group I (control)	1	Plain Mash	4	7.2	7.4	7 4	7 2	6.8	7.2	7 4		B	B	B	—						
	2		4	7 2	7 0	7.0	6.8	7 0	6 8	7 4		B	B	B	—						
	3		4	7 4	7 0	7 2	7 0	6 8	6 8	6.8		B	B	B	—	No cecal droppings passed after May 26.					
	4		3	7.4	6 8	7 4	6 8	7 2	6.8	7 0		B	B	B	—						
	5		3	7.0	7 2	7.2	7.0	7 0	7 0	6 8		B	B	B	—						
	6		2	7.6	6 2	6 8	7 2	7 4	6 8	7 2		B	B	B	—						
Group II	7	40% skim milk powder	3	5.6	X	6 8	5 6	5 8	5 4	6 0	No P.M. samples taken	6 0 B	—	5 4 B	5 2	5 4	—	5 4	—	5 2	—
	8		2	6 8	5 6	5 2	5 2	5 2	5 4	5 6		5 8 B	—	5.2 B	—	B	—	—	—	5 2	—
										5 8											
	9		3	5 4	5.4	5 4	5 4	5.0	5 2	7 0		5 0 B	—	5 4 B	—	5 4 B	—	5 4	—	5 4	—
										6.8											
	10		4	5 0	5 2	5 6	5 2	5 4	5 2	5 2		4 8 B	—	5 0 B	—	5 2 B	—	5 2	—	5 2	—
														5.4							
	11		3	5 2	5 4	5 4	5 4	5 2	5 6	5 4		5.2 B	—	B	—	5 2 B	—	5 2	—		
	12		2	6.2	X	5 2	5 4	5 0	5 4	5 6		5 0 B	—	B	—	—	—	—	—	5 4	—
										5 2											
Group III	13	20% Lactose Mash	1	5.4	5.2	5.6	5 2	5 2	5 6	5 2		5.0	—	B	—	5 2 B	—	—	—	5 2	5.0
	16		4	6.6	5 6	5 4	5 0	5 0	5 4	5.2		5 4 B	—	B	—	5 2 B	—	—	—	5 2	5 0
										5 4											
	14		4	5.4	X	5 4	5 2	6 2	5 4	5 8		5 6 B	—	B	—	—	—	—	—	5.2	—
	17		4	X	5.2	5 4	5.6	5.0	5.2	5 0		5 0 B	—	5 0 B	—	5 0	—	—	—	5 2	—
										4 8											
	15		4	5.6	5.4	7 0	5 0	7 0	5.4	5 8		5 0 B		B	—	—	—	—	—	5.2	—
	18		1	X	5.4	5.2	5.2	5.2	5 2	5.4		B		All birds dead.							

B=Droppings contain blood. When "B" alone appears in a space, it indicates that all cecal droppings contained blood and no normal sample was taken for pH determination. When both B and a pH value appear in a space, it indicates that blood was present in some of the cecal droppings but some normal droppings were present and were sampled.

—=Cecal droppings absent or liquid and mixed with other droppings.

THIRD TRIAL

In this experiment, the protection of chicks against coccidiosis was attempted by feeding them lactose or dry skim milk mixed with their mash. Sixty-eight chicks, six weeks old, were used. On May 20, approximately 50,000 sporulated oöcysts were introduced into the crop of each bird. The chicks were divided into three groups and immedately supplied wth the following rations:

Group I (controls), consisting of twenty-four birds, was fed a plain mash mixture.

Group II, consisting of twenty-two birds, was fed mash containing 40 per cent dry skim milk.

Group III, consisting of twenty-two birds, was fed mash containing 20 per cent lactose.

The dry skim milk contained 50.6 per cent lactose. This made the lactose content of the mashes for groups II and III approximately the same.

Fourteen deaths from chilling occurred during the three days following infection. The number of birds in Group I was thus reduced to twenty and in Groups II and III to seventeen each.

Mash was kept constantly before the birds, no other food being supplied. No preliminary feeding of dry skim milk or lactose before the administration of oöcysts was given.

From two to four birds were placed in each cage. Samples of cecal droppings for pH determinations were collected twice daily. Since there was more than one bird in a cage, it could not be determined from which individual a particular sample originated. This procedure, however, served to show differences between the pH values of cecal droppings of the three groups. When all deposits of cecal droppings from the birds in a cage were of the same physical character, one sample only was taken, otherwise more than one sample was taken. The first samples of droppings were collected on May 21, the day after the birds were inoculated and the feeding of lactose and dry skim milk was begun. The pH determinations and a summary of the effect of the inoculation on the birds are given in tables 3 and 4.

With few exceptions, the pH of the cecal droppings from the birds of Groups II and III was between 4.8 and 5.6, while in Group I, it ranged from 6.2 to 7.4.

A portion of the cecal droppings passed by Groups II and III on the fifth day contained some blood. This was more marked on the sixth day. A slight amount of blood was present in the droppings on the seventh and eighth days, but thereafter they were normal.

TABLE 4

EFFECT OF INOCULATION WITH SPORULATED OÖCYSTS IN THE THIRD TRIAL

Group No.	Mash fed	Number of birds	5th day*	6th day	7th day	8th day	9th day	Total died from coccidiosis	Per cent died from coccidiosis
I Controls	Plain	20	4 B 8 S 3 D	4 B 8 S 8 D	X 2 S 6 D	X 2 S 1 D	X 0 S 0 D	18	90
II	40% Dry skim milk	17	2 B 1 S 2 D	3 B 4 S 6 D	2 B 0 S 2 D	1 B 0 S 0 D	0 B 0 S 0 D	10	58.8
III	20% Lactose	17	2 B 2 S 3 D	3 B 2 S 7 D	2 B 0 S 1 D	0 B 0 S 0 D	0 B 0 S 0 D	11	64.7

D = Died from coccidiosis. Numeral preceding indicates number of birds.
S = General symptoms such as droopiness, inappetence. Numeral preceding indicates number of birds.
1 B = Blood present in less than half of cecal droppings.
2 B = Blood present in more than half of cecal droppings, but not in all.
3 B = Blood present in all cecal droppings, but all droppings not entirely blood.
4 B = Cecal droppings appear to be entirely blood.
X = No cecal droppings passed.
* = No indications of coccidiosis before fifth day.

Blood was discharged profusely from all birds in Group I on the fifth and sixth days. No cecal droppings were passed by these birds after the sixth day.

As shown in table 4, the first deaths from coccidiosis in all three groups occurred on the fifth day. The total mortality from this cause was eighteen, or 90 per cent, in Group I; ten, or 58.8 per cent, in Group II; and eleven, or 64.7 per cent, in Group III.

Besides suffering a lower mortality than the controls, a smaller number of the birds that were fed dry skim milk or lactose exhibited general symptoms and their droppings contained less blood.

The mortality from coccidiosis was relatively high in all groups. This may have been influenced by a chilling the birds received during the first two nights after the experiment was begun and also by the large dose of oöcysts they received. The results demonstrate, however, that feeding chicks mash containing 40 per cent dry skim milk, or 20 per cent lactose, is of considerable benefit in protecting them against artificial infection with sporulated oöcysts of *Eimeria avium*.

FOURTH TRIAL

This experiment was designed to show the value of feeding of dry skim milk or lactose in protecting chicks, kept under conditions approximating those found in the field, against artificial infection with sporulated oöcysts of *Eimeria avium.*

Day-old chicks from a commercial hatchery were transferred directly to clean brooder pens. To avoid natural infection with coccidiosis, no outside runs were provided.

The chicks were divided into three groups of two pens each. To Group I was fed the following mash mixture:

Dry skim milk	40 parts
Wheat bran	10 parts
Yellow corn meal	30 parts
Ground barley	20 parts
Cod-liver oil	2 parts

The mash mixture for Group II consisted of:

Lactose	20 parts
Wheat bran	30 parts
Bone meal	10 parts
Meat scrap	15 parts
Ground barley	15 parts
Yellow corn meal	10 parts
Cod-liver oil	2 parts

For Group III, the controls, the mash was:

Wheat bran	20 parts
Bone meal	5 parts
Meat scrap	15 parts
Yellow corn meal	30 parts
Ground barley	30 parts
Cod-liver oil	2 parts

At the time these experiments were in progress there was no supply of green feed available in our location which was known to be free from contamination with oöcysts, therefore, cod-liver oil was included in the mash to supply vitamin A.

Scratch grain fed to all pens consisted of equal parts of fine cracked yellow corn, steel cut oats, and cracked wheat.

The mash and grain were fed in the proportion of two parts of mash to one part of grain. Fed in this proportion, the nutritive ratio of the

TABLE 5

TABULATED SUMMARY OF FOURTH TRIAL

Group No.	Pen No.	Number of chicks March 5	Mash fed	Method of inoculation	Died from coccidiosis						
					6th day	7th day	8th day	12th day	13th day*	Total	Per cent
I	1	19	Milk powder	250,000 oocysts orally	2	4	2	None	None	8	42 1
II	1	18	Lactose	250,000 oocysts orally	7	5	2	None	None	14	77 7
III	1	25	Plain (control)	250,000 oocysts orally	16	6	None	1	None	23	92
I	2	24	Milk powder	12,500,000 oocysts in soil	None	None	None	None	None	None	None
II	2	23	Lactose	12,500,000 oocysts in soil	1	3	1	None	None	5	21 7
III	2	24	Plain (control)	12,500,000 oocysts in soil	None	None	None	None	1	1	4 1

* No deaths occurred after the thirteenth day.

rations for all groups was 1 to 3.0.* Mash in metal hoppers was kept before the chicks continuously. The amount of mash consumed was determined by placing a weighed amount in the hoppers each morning and weighing out the unconsumed portion on the following morning. The difference in the weights represented the amount consumed during the preceding twenty-four-hour period and served as an index of the amount of grain to feed to preserve the two to one mash and grain ratio.

When the chicks were fourteen days old, inoculations with sporulated oöcysts were made as follows: Approximately 250,000 oöcysts were introduced into the crop of each chick in Pen I of all three groups. In Pen 2 of all three groups was placed a box of sterilized soil to which was added approximately 12,500,000 oöcysts. The grain fed in these pens thereafter was scattered on the soil. It was thought that by this means the chicks could be made to acquire coccidial infection in a more natural manner.

At the time the oöcysts were administered, the number of chicks in the different pens was as follows:

Group I—Pen 1 contained 19 chicks; Pen 2, 24 chicks
Group II—Pen 1 contained 18 chicks; Pen 2, 23 chicks
Group III—Pen 1 contained 25 chicks; Pen 2, 24 chicks

The oöcysts were administered on March 5. The chicks were kept under observation until March 31.

Deaths occurred from coccidiosis on the sixth day after inoculation and continued until March 18, the thirteenth day. There was no sickness nor death after that date. A tabulated summary of results is given in table 5.

In this table, it is seen that the total mortality of birds receiving the oral administration of oöcysts was 92 per cent in the control pen, 77.7 per cent in the lactose pen, and 42.1 per cent in the dry skim milk pen. Such relatively high mortality in all pens is not greater than is to be expected in view of the enormous dose of oöcysts given to each chick. The results demonstrate, however, that feeding chicks mash containing 40 per cent of dry skim milk affords them considerable protection against severe coccidial infection.

The results obtained with lactose were much less satisfactory. The results of preceding experiments indicate that the value of dry skim milk for coccidiosis control lies in the lactose it contains. Therefore, mashes containing 20 per cent lactose and 40 per cent dry skim milk should be equally effective. A possible explanation of the failure of lactose mash in this case is that a sufficient quantity was not consumed. It was observed that much of this mash was scattered in the litter about the feed hopper, while the dry skim milk mash was consumed without waste.

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* The writers are indebted to W. E. Newlon for assistance in compounding the rations.

The difference in the character of the two mashes appeared to be the factor responsible for the waste in one case and lack of waste in the other. The lactose mash contained 30 per cent of bran and 15 per cent of meat scrap. This made a coarse, flaky mixture that was easily scratched out of the hopper. The coarse brown particles of meat scrap appeared to be attractive to the chicks and in their efforts to pick them out the lighter part of the mash was thrown out on the floor and lost. The dry skim milk mash, on the other hand, was a uniform, nearly white, somewhat adhesive mixture of fine texture which did not tempt the chicks to pick through it and which was, therefore, consumed without waste.

The only serious mortality resulting from feeding grain on soil with which oöcysts had been mixed was that of 21.7 per cent in the lactose pen. No chicks died in the dry skim milk pen and only 4.1 per cent died in the control pen. The explanation of the slight degree of infection among the control chicks is evidently the failure on their part to ingest enough oöcysts to produce disease, since it is definitely known that the oöcysts were present in the soil and that the chicks were susceptible.

FIFTH TRIAL

The method of procedure followed in this experiment, with the exception of the changes noted below, was the same as in the immedately preceding one. Pens in which infecton was attempted by means of feeding grain in soil contaminated with sporulated oöcysts were omitted because of the uncertainty of infecting chicks by this method. The mash mixtures were the same, except that wheat shorts were substituted for wheat bran and the meat scrap was sifted through a fine screen. The purpose of these changes was to provide as nearly as possible the same degree of fineness in all mashes. It was thought that by this means the temptation for the chicks to pick over the mash would be removed and the wasting of mash from this cause thereby avoided.

One hundred and fifty chicks, forty-eight hours old, were divided into three pens of fifty chicks each. They were given their first feed when seventy-two hours old. Pen 1 received the 40 per cent skim milk powder mash; pen 2, the 20 per cent lactose mash, and pen 3, the controls, the plain mash.

During the first five days, the ration consisted entirely of mash which was before the chicks at all times. At this point, the sudden onset of a period of cold, damp weather had an unfavorable effect on all of the chicks, but was more serious among those in Pens 1 and 2 than in the control pen. This appeared to be due to the fact that the litter in Pens 1 and 2 became damp, while that in Pen 3 remained dry. This dampness resulted from the watery consistency of the droppings from the chicks fed dry skim milk and lactose. It was thought desirable, therefore, to reduce consumption of these mashes until the weather moderated by feeding scratch grain in addition to the mash. Scratch grain was fed twice daily for ten days. The chicks in all pens now appeared equally vigorous. From this time until the termination of the experiment, grain was fed in the morning only and the amount supplied restricted to one-third that of the mash consumed.

On April 30, when the chicks were eighteen days old, 1000 sporulated oöcysts were introduced into the crop of each chick. Pen 1 now contained forty-two chicks; Pen 2, thirty-nine chicks; and Pen 3, forty-two chicks.

Deaths from coccidiosis began on the sixth day after inoculation and continued through the seventh and eighth days.

A summary of the results is given in table 6:

TABLE 6

TABULATED SUMMARY OF FIFTH TRIAL

Pen No.	Ration	Number of chicks inoculated	Total died from coccidiosis	Per cent died from coccidiosis	Average weight per chick 32 days old	Average daily mash consumption per chick
1	Skim milk powder mash..............	42	1	2.3	185.5 gm.	12.5 gm.
2	Lactose mash	39	3	7.7	133.3 gm.	10.9 gm.
3	Plain mash (control)	42	10	23.8	146.9 gm	12.9 gm.

The results, as shown in this table, clearly demonstrate the effectiveness of dry skim milk in combatting coccidial infection. Bloody droppings were passed by several birds in Group 1 in addition to the one which died, but none of them were visibly sick.

The results obtained with lactose were less satisfactory than those with dry skim-milk, but still demonstrated that the birds to which it was fed were given considerable protection against coccidiosis. As recorded in the table, it was found that the mash consumption in this pen was less than in either of the other two. This is probably a factor responsible for the difference in the effectiveness against coccidiosis afforded by the dry skim milk and lactose mash mixtures.

Another factor that is probably in part responsible for the greater effectiveness of dry skim milk against coccidiosis is the superior food value of this milk product as indicated by the increased growth made by the chicks in Group 1.

This increase in weight amounted to 38.6 grams a chick, or 26.2 per cent more than was made by those in the control pen, which were fed the plain mash, in spite of the fact that the latter consumed 0.4 grams more mash per chick daily than those receiving the dry skim milk mash. The chicks which were fed the lactose mash consumed 12.8 per cent less than those receiving dry skim milk mash and, therefore, attained the least growth.

SUMMARY AND CONCLUSIONS

The results of the series of five experiments were uniform in demonstrating that chicks were afforded a considerable degree of protection against coccidial infection when a sufficient amount of lactose or dry skim milk was added to their diet. In the trials carried out under laboratory conditions, this was accomplished equally well by the individual administration of two 1-gram doses of lactose to each bird daily at an interval of about eight hours or by feeding chicks continuously with mash containing 20 per cent lactose or 40 per cent dry skim milk. In the trials carried out under field conditions, however, the results obtained from the use of skim-milk powder were superior to those obtained from the use of lactose. This was due, at least in part, to the fact that the chicks did not relish the mash mixture containing lactose and, therefore, consumed less of this mash than of that containing dry skim milk. The relatively greater increase in weight of the chicks fed on dry skim milk indicated that the superior food value of this material was also at least in part responsible for the benefit derived from its use.

The results of these experiments, confirm those described in the preceding paper in showing that when sufficient lactose or dry skim milk is fed to chickens, the hydrogen ion concentration of the cecal contents can be kept within a range of 4.4 to 5.6. It is thought that this degree of acidity may be sufficient to injure or destroy the sporozoite or merozoite forms of *Eimeria avium* and that serious harm from the infection is thereby prevented. However, both merozoites and oöcysts were found in the droppings of birds inoculated with sporulated oöcysts and treated with lactose or dry skim milk even though the birds showed no visible signs of sickness after the inoculation. This is evidence that at least a part of the sporozoites released from the sporocysts were unharmed and invaded the cells of the cecal mucosa where both the sexual and asexual cycles of development were completed. A possible explanation of this is that the dose of sporulated oöcysts given was too large to be entirely overcome and, therefore a portion of the sporozoites escaped. Another possible explanation is that the acidity in the ceca was more destructive to the merozoites than to the sporozoites. In such a case, the invasion of the epithelial cells by the sporozoites and the completion of the developmental cycles within the cells would be unhindered. The merozoites, however, upon emergence from the epithelial cells into the acid cecal contents would be destroyed and further development of disease arrested. On this basis, the appearance of blood in the droppings and death on the fifth and sixth days after inoculation of some of the birds which were fed lactose or skim-milk powder could be ascribed to the tissue damage resulting from the initial invasion with sporozoites. The destruction of the merozoites, however, prevented further development of disease in the birds which were not fatally injured by the sporozoite invasion.

This explanation would not apply to the failure of lactose feeding in the last two coccidiosis control trials, to afford the chicks as high a degree of protection against coccidial infection as was given by dry skim milk. This, as previously pointed out, was probably due in part to the difference in amount of consumption of the two mash mixtures by the chicks (12.8 per cent less of lactose) and also in part to the superior food value of the skim-milk powder.

The fact that feeding chickens mash containing 40 per cent dry skim milk not only protected them against coccidial infection, but also stimulated rapid growth indicates that this would be a valuable practice in the prevention and control of outbeaks of the disease on poultry farms.

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SECONDARY SEX CHARACTERS IN ASPARAGUS OFFICINALIS L.

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INTRODUCTION

Common asparagus is normally dioecious. Casual observations in the field reveal no striking differences between the two sexes in their vegetative characters. Careful studies, however, show that there are significant quantitative differences between staminate and pistillate individuals.

Secondary sex characters in plants are far less striking than they are in animals. It is probably for this reason that they have received so little attention.

Cowles[1] calls attention to the fact that "immediately after flowering it often is possible to distinguish at some distance pistillate from staminate mulberry trees by their much smaller leaves, as though the constructive material in the former were utilized chiefly in fruit development, and in the latter, in leaf development." Later in the season, the leaves are equally large on both pistillate and staminate individuals.

Guinier[3] states that dioecism in the case of *Pinus montana* and *P. sylvestris* is accompanied by a certain vegetative dimorphism. In purely staminate individuals, the branches terminating in inflorescences have the cones distributed over the greater part of the new shoots, a relatively small area being left at the apex for the short, leaf-bearing twigs. After these male cones have fallen, there is left a long bare section at the base of each new shoot, the leaves forming a tuft at the tip of the shoot. In purely pistillate individuals, the branches terminating in inflorescences are almost entirely covered with leaves. It is stated further that in *Pinus montana,* subsp. *uncinata* (in the Pyrenees), the staminate individuals, when compared with pistillate, are seen to have wider spreading, somewhat intertwined, branches, less abundant foliage and a more irregular and spreading crown.

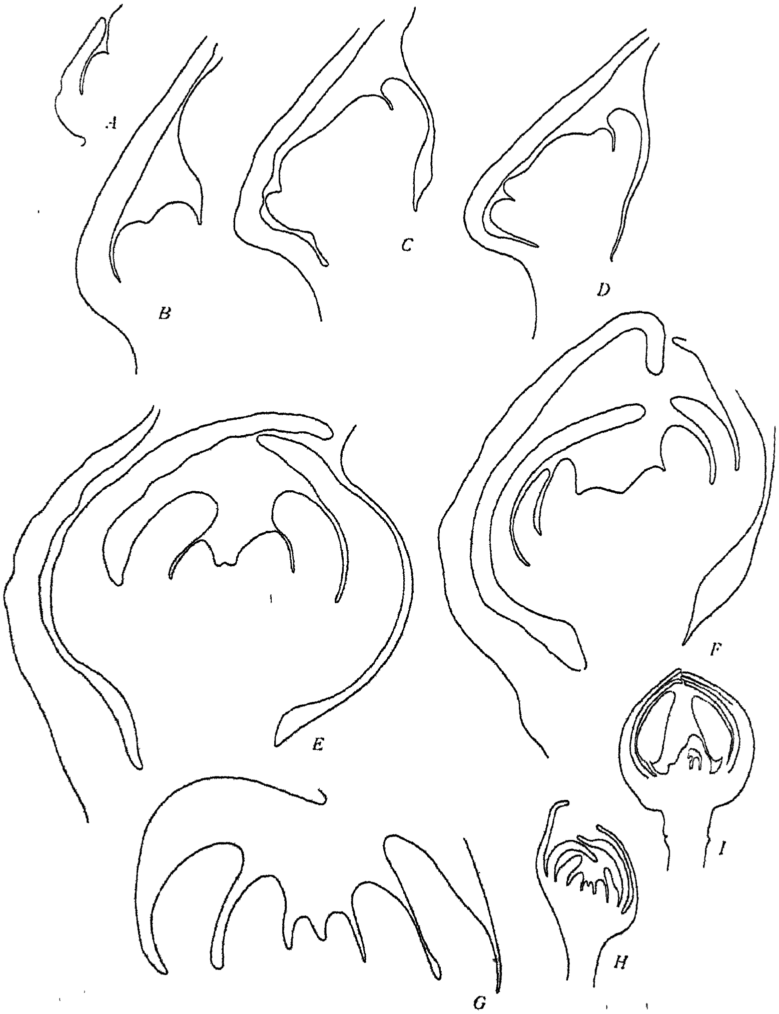


Fig. 1.—Early stages in the development of an asparagus flower (staminate).

Hemp (*Cannabis sativa* L.) is dimorphic in its vegetative as well as its floral characters. McPhee[4] describes the differences between male and female individuals as follows:

"Staminate plants: More slender and taller than carpellate plants because of the rapid elongation of the internodes just prior to anthesis; terminal inflorescences with practically no leaves; flowers normally with five sepals and as many anthers; much shorter life than the carpellate type.

Carpellate plants: More vigorous but shorter than the staminate type; terminal inflorescence leafy; broad crown of leaves; flowers with perianth but no vestige of stamens; weight at maturity about twice that of the staminate type; longer life."

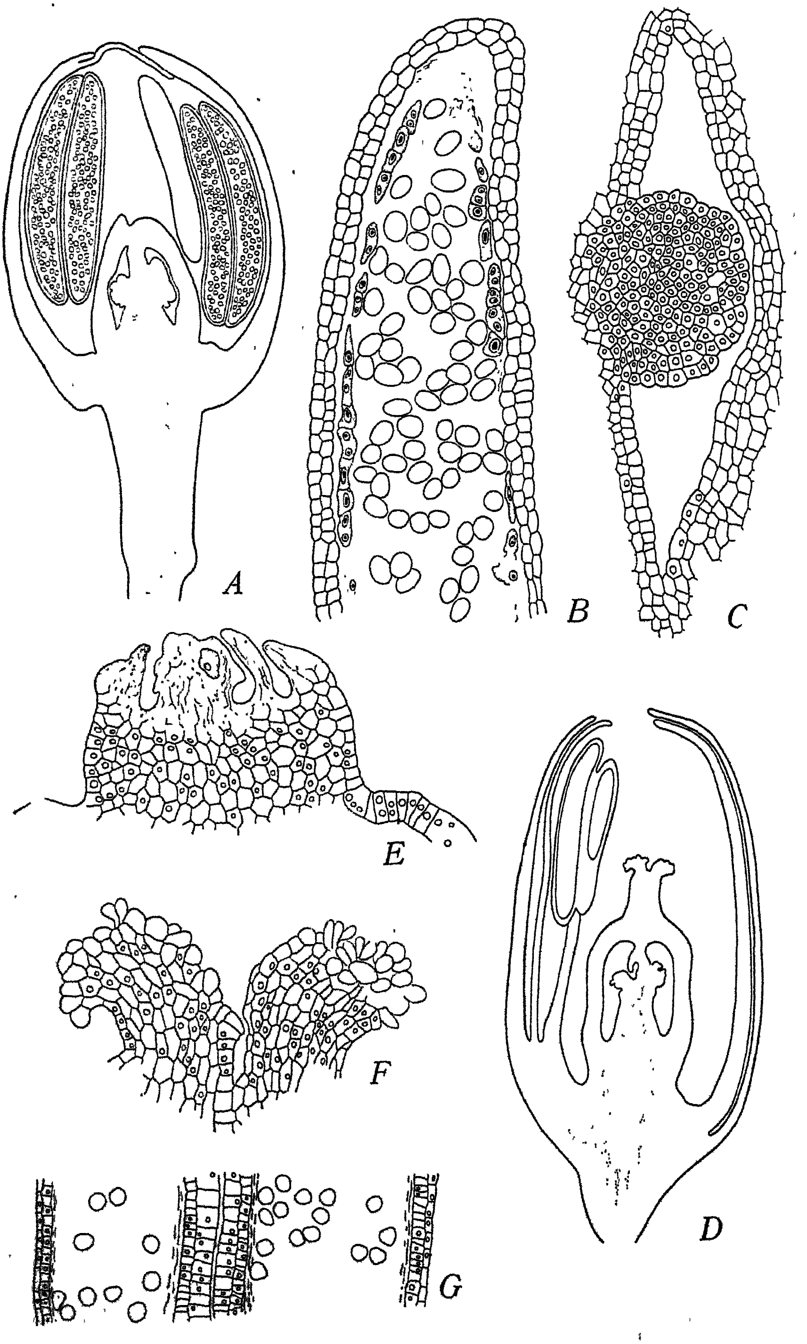
Rosa[6] finds that the male spinach plants send up their seed stems and bloom from one to two weaks earlier than the female. The male plants die from two to four weeks after the beginning of anthesis while the female plants continue blooming for a period of eight weeks or more.

In *Merculialis annua* L., according to Yampolsky,[9] "the female inflorescences are borne in clusters in the axils of the leaves, while the male inflorescences are borne in interrupted spikes which surpass the leaves."

Floral Development.—It is not our purpose here to describe fully the floral development in asparagus. A brief statement will suffice to make clear the bearing it has on the main subject matter of this paper.

The first evidence of the flower is a slight rounded protuberance in the axil of a scale leaf (fig. 1, A). This broadens and becomes slightly elevated at the rim. The primordia of the outer perianth segments are the first to appear; these are followed in turn by the primordia of the inner whorl of perianth segments, the outer whorl of stamens, the inner whorl of stamens, and last by the carpels. The stages in the development of a staminate flower are shown in fig. 1. The early stages of development of staminate and pistillate flowers are indistinguishable.

Fig. 2.—Developmental stages of two different staminate flowers (A, B, and C) and (D, E. F, and G), showing abortion of pistil. A, median lengthwise section of staminate flower; B, portion of single locule of anther, showing wall cells, disintegrating tapetal cells, and pollen grains; C, ovule of flower shown in A and B; note that in this particular flower, the megaspore mother cell had not become differentiated in the nucellus, although pollen grains are mature; D, median lengthwise section of another staminate flower; E, in this flower, the megaspore mother cell had become differentiated by the time the pollen grains were mature, but the surrounding nucellar tissue and integuments showed disintegration; F, stigma of same flower; G, section of anther.


A
B
C
E
F
D
G

In young staminate flowers, the rudimentary pistil may have a shape typical of that of pistillate flowers of the same age, but it fails to attain normal size. The absence or very weak development of the style and stigma is characteristic of staminate flowers; it may have locules in the ovary, and ovules may begin development, but they fail to reach maturity. The ovule of the staminate flowers seldom

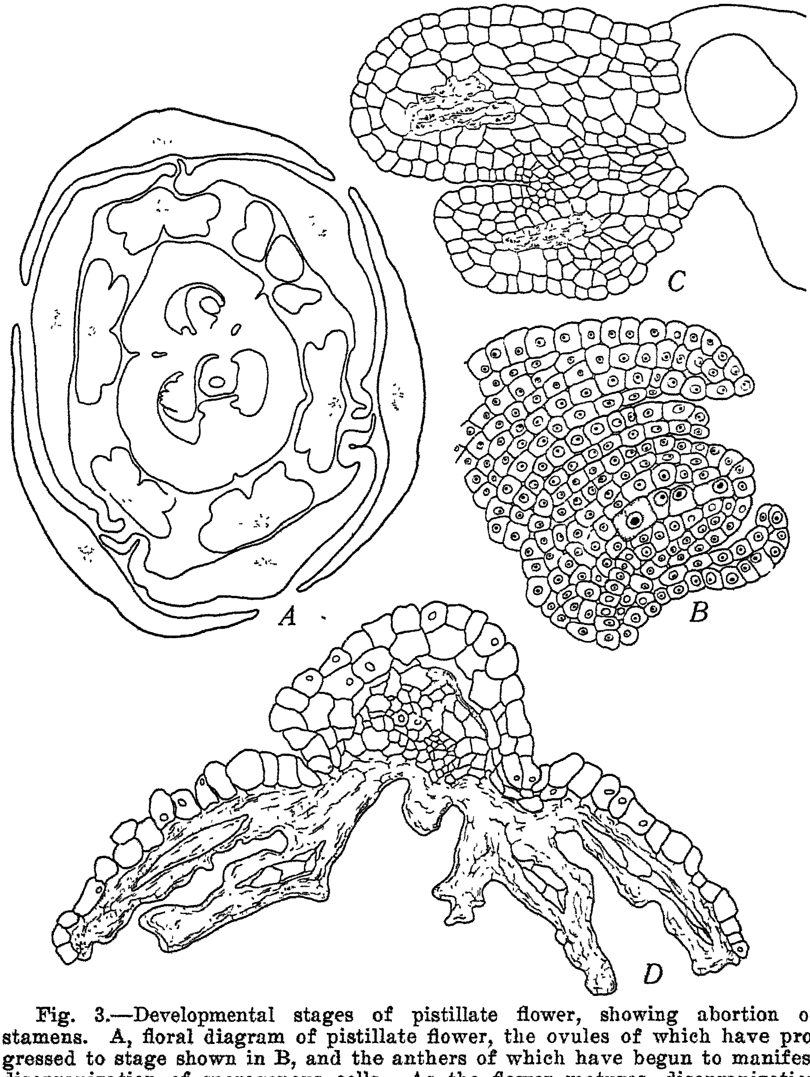


Fig. 3.—Developmental stages of pistillate flower, showing abortion of stamens. A, floral diagram of pistillate flower, the ovules of which have progressed to stage shown in B, and the anthers of which have begun to manifest disorganization of sporogenous cells. As the flower matures, disorganization of anther tissue proceeds, finally attaining the condition shown in D.

develops further than the primary archesporial stage; the integuments seldom attain a size sufficient to enclose the ovule. Disorganization of the ovule begins before the anther of the same flower has reached maturity (fig. 2). Young pistillate flowers may develop for a time stamens similar to those of the purely staminate flowers, but the anthers do not attain normal size, the filaments are short, and the immature pollen mother cells disintegrate. Shortly after, the entire anther shrivels (fig. 3).

It will thus be observed that all asparagus flowers in the primordial stages bear both sets of sex organs, and hence are apparently potentially hermaphroditic. During their subsequent development there is, except in rare cases, an abortion of either the male or female sex organs. The degree of abortion varies. There is no evidence of transmutation of pistils into stamens and of stamens into pistils.

Sex Intergrades.—Sex intergrades occur in a great many plants (Yampolsky[9]) among which should be included *Asparagus officinalis*. Normal male flowers bear six well-developed stamens and a single rudimentary pistil (fig. 4). Normal pistillate flowers have a single well-developed pistil and six rudimentary stamens (fig. 4). Occasionally, hermaphroditic flowers are found. Our observations show that hermaphroditism in asparagus is very rare, at least under Californian conditions, and is limited to a relatively few flowers on plants that are predominately staminate (fig. 5).

Regarding asparagus, Norton[6] states: ''Hermaphrodite plants occur now and then, but so far in our experiments can not be considered a factor in seed production. In flowers of the typical female plant, the rudiments of stamens exist, but the writer has never seen one developed sufficiently to even suggest the possibility of self-pollination. On the other hand, the male plants often show a well-developed ovary with style and stigma and sometimes even a typical stigmatic surface. The great majority of male flowers, however, lack a well-developed ovary, the rudiments being about half the size of the normal ovary of the female flower and lacking any stigmatic development, the style often being completely abortive. The hermaphrodite plants mentioned above are always of the male type, the flowers being for the greater part pure male in that they lack the complete and functionary ovary. In one wild plant examined the flowers at the extremities of the branches were typically female with well-developed stigma and abortive anthers. Another hermaphrodite plant which produced seed that would germinate and make healthy, vigorous plants had many flowers whose ovaries lacked the stigmas. The berries on these hermaphrodite plants are very small and rarely have more than one seed in them. The seeds are usually peculiar in that the seed coats are not entirely developed. The seeds appear mottled black and white, varying from the white of the uncovered endosperm in the smaller seeds to well-covered, entirely black seeds in which the coats have had their normal development and have completely covered the endosperm. These small seeds make weak plants and in many cases abnormal ones, but the larger, better developed seeds make healthy seedlings of normal type."

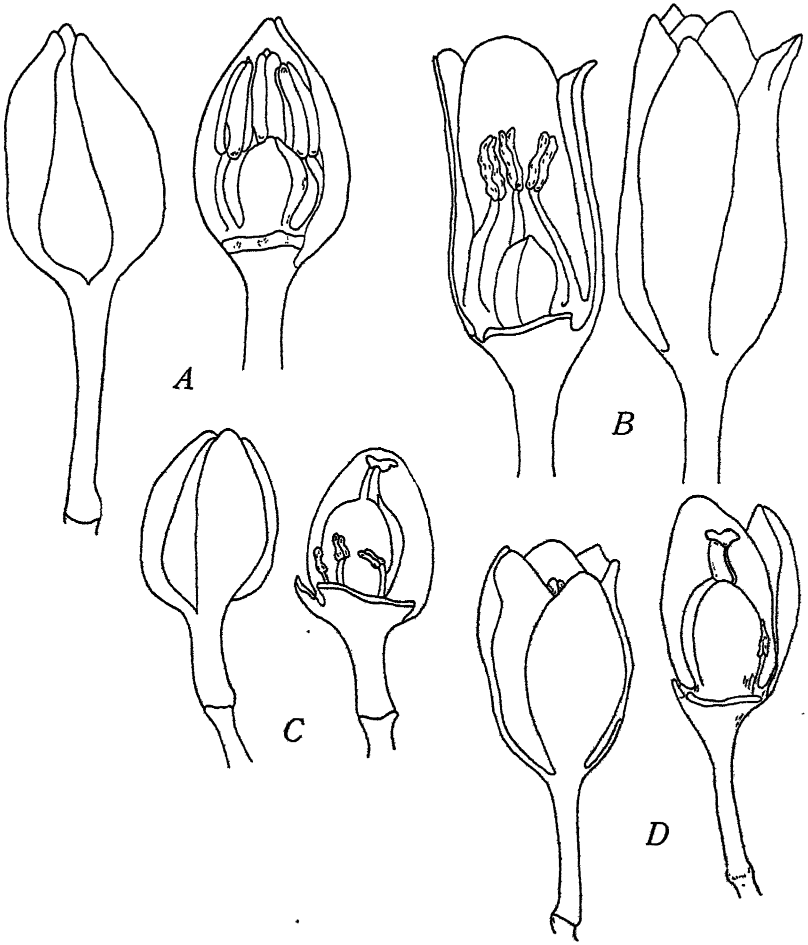


Fig. 4.—Strongly staminate and strongly pistillate flowers in external view, and with a part of the perianth segments removed. A, strongly staminate flower before anthesis; B, the same, after dehiscence of anthers; C, strongly pistillate flower, before anthesis; D, the same, when the stigma is receptive.

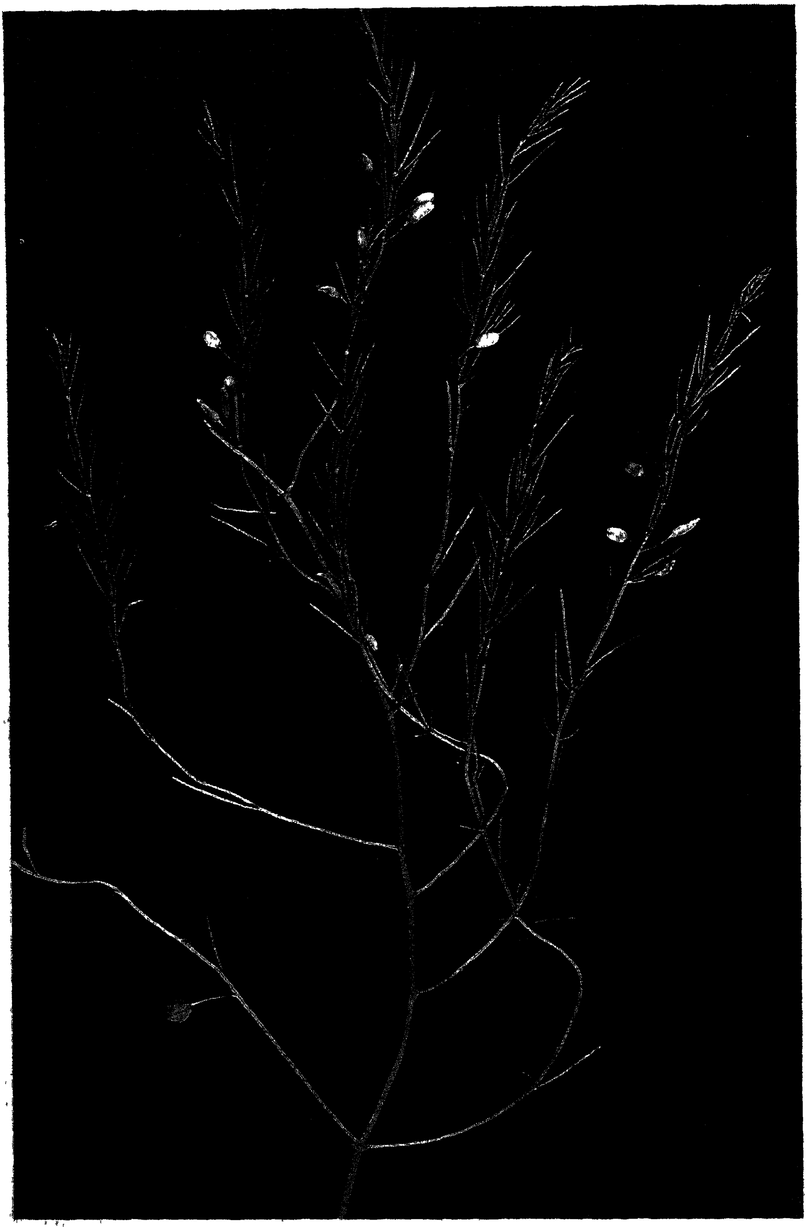


Fig. 5.—Asparagus shoot collected November 15, 1924, Davis, California. The majority of the flowers on this plant were strongly staminate, but occasionally toward the base of the shoots, a few hermaphroditic flowers occurred. Observe the single berry.

The following forms of individual flowers in asparagus have been observed: (1) strongly pistillate, (2) weakly pistillate, (3) hermaphroditic, (4) weakly staminate, and (5) strongly staminate. In "strongly pistillate" flowers, the ovary is well developed, the style is long, and the stigmatic surface plainly visible; whereas, the stamens are represented by the merest traces of atrophied tissue (fig. 4). In "weakly pistillate" flowers, the ovary is somewhat smaller than in the preceding case, but ovule-bearing, the style is short, the stigmatic surface is not so well defined, and the form of the anthers may be observed, although no pollen is produced, and the anthers soon wither. In true hermaphroditic flowers, both pollen-bearing anthers and ovule-bearing pistils are developed. Only a very small percentage of true hermaphroditic flowers have been observed among the hundreds of flowers examined in Californian asparagus fields. In "weakly staminate" flowers the stamens are short, but functional, and the ovaries are quite large and bear a short style but no ovules. In "strongly staminate flowers the stamens are well developed and normal in size, the pollen grains are functional, and the only evidence of the pistil is a very small conical body, without a style, in the center of the flower (fig. 4).

It has been observed that the same plant may bear both strongly and weakly pistillate flowers, or strongly and weakly staminate flowers.

Sex Ratio in the Fields.—The ratio of staminate and pistillate plants in commercial fields is shown in the accompanying table. This ratio was determined by walking down the asparagus rows and recording the sex of each plant.

TABLE 1

RATIO OF STAMINATE AND PISTILLATE PLANTS IN THE FIELD

Ranch	Location	Bed set	Date counted	Number of plants observed	Per cent staminate	Per cent pistillate	Per cent plants not in bloom
Cal. Packing Corp.	Ryer Island	1921	5/5/23	97	50.5	48.4	1.1
Cave & Patterson.	Holland Land Tract	1922	6/9/23	944	47.3	52.3	0.4
Montezuma Ranch	Collinsville	1921	7/6/23	137	51.8	48.2	0
R. J. Grahams	Walnut Grove	1922	8/9/23	233	50.2	49.8	0
Hamilton Bros	Tyler Island	1921	9/8/23	185	48.7	51.3	0

The data show that in the commercial asparagus fields there are virtually equal numbers of staminate and pistillate individuals.

Expression of Sex of Seedling Plants.—In the eastern states asparagus plants seldom bloom until the second year from seed, but in California a large percentage of plants flower the first year.

In the asparagus nursery on the University Farm, Davis, California, the blooming or fruiting dates of many seedling plants were recorded. Seed was planted February 28 and March 8.

TABLE 2

TIME OF SEX EXPRESSION OF SEEDLING PLANTS

Variety	Number of plants observed	Sex	Plants in bloom or fruit (1923)—Dates										
			7/2	7/7	7/11	7/13	7/16	8/2	8/4	8/7	8/11	8/27	9/3
Palmetto..........	2400	S*	15	26	37	88	127	173	201	398	450	507	530
Palmetto..........	2400	P*	0	0	0	0	0	3	17	47	86	120	213

* S=staminate; P=pistillate.

The above table shows that there is a tendency for staminate plants to express their sex much earlier in life than pistillate plants. Many plants do not flower the first season, but of those that do, by far the larger percentage are staminate. The last counts in the field were made on September 3, and by this date in a total population of 2400 plants 743 or 31 per cent had expressed their sex. Before the plants were killed by frost many more came into bloom. When the crowns were dug during the following winter, 482 additional plants were bearing fruit, making a total of 695 pistillate plants out of 2400 which expressed their sex the first year. If half of the plants are pistillate then approximately 58 per cent of these bloomed the first season in the nursery. Many more staminate plants came into bloom after September, but it was difficult at the time the crowns were dug to identify them as only the pedicels of the flowers remained.

Comparison of Staminate and Pistillate Seedling Plants.—Each new shoot which arises on the crown during the first year from seed is usually larger than the preceding. The first four secondary shoots so far as observed were never flower-bearing, but in a few individuals, the fifth secondary shoot did produce flowers. In staminate plants, the first flower-bearing shoot varied from the 5th to the 11th (average, 7.2 for the 149 plants observed); in pistillate plants the first flower-bearing shoot varied from the 5th to the 14th (average 8.5 for the 80 plants observed). These data were taken from August 8 to 11 on the Palmetto variety. Thus it is seen the first flower-bearing shoot usually appears earlier in the life of the staminate than in the pistillate plant. Measurements made on the above two groups of plants (149 staminate and 80 pistillate), show that the average height of the first flower-bearing shoot of staminate plants is 47.8 cms., while the average height of the first flower-bearing shoot of pistillate plants is 62.4 cms., a difference in height of 14.6 cms., in favor of the pistillate stalks.

Top Growth of Staminate and Pistillate Plants the Year Crowns Are Set (1924). The relative vigor of staminate and pistillate plants, as indicated by the top growth made by the plants the year the crowns are set, is shown in table 3.

TABLE 3

STAMINATE AND PISTILLATE PLANTS—COMPARATIVE TOP GROWTH THE FIRST YEAR (1924)*

STAMINATE PLANTS

Row No.	Number of crowns	Average number of stalks per plant		Average total height per crown (inches)	Average height per stalk (inches)	Total weight green tops (lbs.)	Average weight green tops per crown (lbs.)
(1)	(2)	May 25 (3)	Nov. 8 (4)	May 25 (5)	May 25 (6)	Nov. 8 (7)	Nov. 8 (8)
1	116	2.5	10.5	84.5	33.8	112.00	.97
3	120	2.9	....	66.3	22.8	87.75	.73
5	121	2.9	....	74.9	25.8	84.75	.70
7	118	2.2	....	67.2	30.4	112.75	1.00
15	233	....	8.0	....	....	142.00	.61
17	154	....	9.7	....	....	122.00	.79
19	93	....	10.4	....	....	103.50	1.11
21	81	....	10.8	....	....	93.50	1.15
31	120	3.0	....	73.0	24.3	104.75	.87
33	119	2.9	....	62.3	21.5	88.75	.75
35	118	2.5	....	50.9	20.4	88.75	.75
37	116	2.5	....	74.1	29.6	101.25	.87
45	236	....	6.7	....	....	108.50	.48
47	156	....	8.6	....	....	121.50	.78
49	93	....	10.2	....	....	90.50	.97
51	79	....	8.8	....	....	66.00	.84
61	120	1.1	....	45.2	41.1	89.75	.74
63	117	1.7	....	44.3	26.1	82.75	.71
65	120	1.6	....	41.0	25.6	87.75	.73
67	118	2.1	....	63.7	30.4	101.25	.86
Average.....		2.37 ±0.122	9.3 ±0.338	63.3 ±2.8	27.6 ±1.14	99.5 ±2.6	0.82 ±0.024

* Analysis of these data shows that the differences between staminate and pistillate plants as expressed in columns 3, 4, and 5 are significant, whereas those of columns 6, 7, and 8 are not significant.

TABLE 3—(*Continued*)

STAMINATE AND PISTILLATE PLANTS—COMPARATIVE TOP GROWTH THE FIRST YEAR (1924)*

PISTILLATE PLANTS

Row No.	Number of crowns	Average number of stalks per plant		Average total height per crown (inches)	Average height per stalk (inches)	Total weight green tops (lbs.)	Average weight green tops per crown (lbs.)
		May 25	Nov. 8	May 25	May 25	Nov. 8	Nov. 8
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
2	118	2.3	...	62.0	26.9	86 75	.73
4	119	1.9	...	55.6	29 3	74 75	.63
6	119	2.6	...	70.5	27 7	73.75	.62
8	118	1.6	...	53.3	33.3	98.75	.84
16	235	...	4 3	...	...	143.00	.63
18	154	...	6.5	...	...	129.00	.84
20	95	...	6.8	...	...	89.00	.94
22	76	...	7.2	...	...	85.00	1.11
32	117	2.0	...	57.2	28.6	83.75	.71
34	120	1.7	...	51.3	30 2	78.75	.66
36	119	1 9	...	52.0	26.8	79.75	.67
38	116	1.7	...	55.5	32.6	71.75	.62
46	234	...	4.5	...	...	114.50	.48
48	153	...	6.2	...	...	136.50	.89
50	92	...	6.4	...	...	80.00	.87
52	78	...	7.3	...	...	76.00	.97
62	117	1.7	...	45 7	26.8	95.75	.82
64	115	1.6	...	43.5	27.2	86.75	.75
66	114	1.5	...	44.2	29.4	86.25	.76
68	108	1.7	...	52.8	30.5	101.25	.94
Average.....		1.88 ±0.062	6.15 ±0.002	53.6 ±1.43	29.1 ±0.66	93.50 ±3 2	.77 ±0.024

* Analysis of these data shows that the differences between staminate and pistillate plants as expressed in columns 3, 4, and 5 are significant, whereas those of columns 6, 7, and 8 are not significant.

Table 3 shows that during the first season staminate plants produce a greater number of stalks, and a greater total height of stalks to the crown, but that there is no significant difference between staminate and pistillate as to average height of the stalk, total green weight of tops or average green weight of tops. A large percentage of the weight of the pistillate plants is comprised in the berries; the weight of these was not determined separately. If the weight of berries is subtracted from the green weight of tops it appears that the food-manufacturing surface of staminate plants exceeds that of the pistillate. It is to be expected therefore that a greater quantity of reserved food would be stored in crowns of staminate than in those of pistillate plants, and that the yield of shoots the following season

TABLE 4

STAMINATE AND PISTILLATE PLANTS—COMPARATIVE TOP GROWTH THE SECOND YEAR (1925)*

STAMINATE PLANTS

Row No. (1)	Number of crowns (2)	Average number of stalks per plant (Oct. 10, 1925) (3)	Total weight of green tops (lbs.) (4)	Average weight of green tops per crown (lbs.) (5)
1	116	10.5	294	2.5
3	118	9.1	298	2.5
5	117	10.8	309	2.6
7	118	6.9	360	3.1
15	233	4.8	410	1.8
17	153	7.6	394	2.6
19	93	11.6	375	4.0
21	81	14.2	312	3.9
31	118	12.0	403	3.4
33	118	8.7	340	2.9
35	118	9.7	370	3.1
37	116	7.8	339	2.9
45	236	5.3	350	1.5
47	155	7.7	404	2.6
49	93	9.2	294	3.1
51	79	10.6	235	3.0
61	119	9.1	318	2.7
63	116	6.9	325	2.8
65	119	8.3	312	2.6
67	116	7.4	285	2.5
Average....		8.9 ±1.57	336.4 ±28.2	2.8 ±0.36

PISTILLATE PLANTS

			With berries (a)	Without berries (b)	With berries (a)	Without berries (b)
2	117	6.2	311	193	2.7	1.7
4	115	6.0	235	146	2.0	1.3
6	116	6.0	250	155	2.2	1.3
8	117	4.7	321	198	2.7	1.7
16	235	3.8	439	272	1.9	1.2
18	154	5.7	382	237	2.5	1.5
20	94	6.1	270	167	2.9	1.8
22	76	7.7	245	152	3.2	2.0
32	117	5.9	249	154	2.1	1.3
34	119	5.7	300	186	2.5	1.6
36	119	5.3	255	158	2.1	1.3
38	116	5.4	282	175	2.4	1.5
46	234	3.1	389	241	1.7	1.0
48	151	4.4	425	264	2.8	1.7
50	92	5.5	248	154	2.7	1.7
52	78	7.1	160	99	2.1	1.3
62	116	4.9	295	183	2.5	1.6
64	115	4.8	266	165	2.3	1.4
66	113	4.9	222	138	2.0	1.2
68	107	4.8	229	142	2.1	1.3
Average.....		5.4 ±0.68	288.7 ±47.8	178.9 ±29.6	2.4 ±0.28	1.5 ±0.18

* Analysis of these data shows that the differences shown in columns 4a and 5a are not significant but that all other differences are significant.

would be greater. Table 6 shows that staminate plants do out-yield the pistillate at least the first season.

Top Growth of Staminate and Pistillate Plants the Second Year After Crowns Are Set (1925).—In early October, 1925, the top growth from the same rows considered in 1924 was harvested. At this time, the leaves were beginning to show a slight tinge of yellow. The plants were cut about two or three inches above the ground line and weighed within a few minutes afterwards, so that the amount of water lost was negligible. As in 1924, the number of stalks produced by staminate plants exceeds that from pistillate plants (table 4). When the weight of berries is added to that of the green tissue, there is no significant difference between staminate and pistillate plants as to the green weight of the top growth. But, if the weight of berries is deducted from the total weight of the plants, and a comparison made of green tissue, it is seen that the amount of this tissue produced by staminate plants greatly exceeds that produced by pistillate. The figures given in column 4*b* of table 4, are based upon the weight of mature berries harvested from row 8, in which it was found that the berries constituted 32 per cent of the total weight of the plants. Approximately 50 per cent of the weight of berries was seed. The berries were picked off by hand, care being taken to avoid stripping the "needles" from the plant.

Yield of Spears from Staminate and Pistillate Plants.—Experiments by Green,[2] performed on a small scale, seem to show that the staminate asparagus plants out-yield the pistillate under conditions as they exist in Ohio. Results obtained by Green are given in the following table:

TABLE 5

PRODUCT FROM FIFTY PLANTS EACH, STAMINATE AND PISTILLATE

	Fifty staminate plants, ounces	Fifty pistillate plants, ounces
First period, ten days	37	21
Second period, ten days	104	68
Third period, ten days	266	164
Fourth period, ten days	203	154
Total for the season	610	407

The staminate plants yielded 50 per cent more than the pistillate plants during the season. The superiority of the staminate plants is greater during the early part of the cutting season than during the late. Green concluded that fruit production makes a greater demand for food than does the formation of stamens, and that it is for this reason that staminate plants are able to produce a greater growth of spears than pistillate.

Tompson[7] reports experiments with the Washington strain of asparagus at the Market Garden Field Station, Lexington, Mass., which show that the staminate plants produce more spears, but

TABLE 6

COMPARISON OF YIELD OF STAMINATE AND PISTILLATE PLANTS (1925)

Row No.	Sex	Average weight per crown when planted (gms.)	Number of crowns	Harvested Feb. 25 to Mar. 9		Harvested Feb. 25 to April 1				
				Total number of spears	Total weight of spears (gms.)	Total weight of spears	Average number of spears per crown	Total weight of spears per row (gms.)	Average weight of spears per crown (gms.)	Average weight of a single spear (gms.)
1	S	231	116	9	154	335	2.88	5543	47.8	16.66
2	P	206	118	5	94	196	1.66	3510	29.7	18.61
5	S	229	121	23	356	311	2.56	5185	42.8	16.27
6	P	232	119	11	157	206	1.73	3631	30.5	18.04
7	S	....	118	23	607	336	3.02	8229	74.0	25.72
8	P	....	118	1	55	199	1.61	5863	49.7	29.43
15	S	231	233	57	1145	570	2.44	10447	44.7	18.32
16	P	242	235	20	412	340	1.49	7606	33.4	22.35
17	S	164	154	45	942	445	2.89	8540	55.2	19.14
18	P	222	154	11	277	323	2.10	6273	40.7	19.43
19	S	189	93	40	865	362	3.89	7591	81.5	20.98
20	P	194	95	7	180	187	1.97	4638	48.8	24.78
21	S	161	81	22	440	276	3.41	5440	67.1	19.67
22	P	230	76	14	397	196	2.58	4568	60.0	23.23
31	S	205	120	43	394	423	3.53	8281	68.8	19.56
32	P	185	117	32	653	279	2.38	5598	47.8	20.13
35	S	165	118	32	565	332	2.81	5928	50.3	17.85
36	P	188	119	14	255	214	1.80	4055	34.0	19.25
37	S	150	116	54	1255	399	3.44	7865	67.9	19.72
38	P	115	116	26	647	234	2.02	5205	44.9	22.22
47	S	191	156	44	880	464	2.97	9143	58.5	19.68
48	P	159	153	33	752	346	2.26	8358	54.7	24.12
49	S	134	93	32	690	366	3.93	7007	75.4	19.65
50	P	129	92	11	310	167	1.82	4206	45.7	25.18
51	S	59	79	19	422	202	2.56	4152	52.5	20.53
52	P	155	78	25	597	216	2.77	4942	63.4	22.87
61	S	153	120	43	775	320	2.66	5815	48.4	18.15
62	P	186	117	12	229	244	2.08	5129	43.8	20.99
65	S	177	120	43	800	305	2.54	5751	47.9	18.86
66	P	172	114	7	100	197	1.73	3878	34.0	19.75
67	S	185	118	52	962	386	3.27	7367	62.5	19.07
68	P	187	108	22	605	204	1.89	4838	44.8	23.66

*An analysis of these data, using Students Method, shows that the results here given are significant.

Summary of Table 6

	Staminate	Pistillate
Total number of crowns	1949.00	1922.00
Total number of spears harvested from Feb. 25 to Mar. 9	581.00	251.00
Average number of spears harvested per crown from Feb. 25 to Mar. 9	0 29	0.13
Ratio of number of spears harvested from Feb. 25 to Mar. 9	2.32	1.00
Total number of spears harvested for season Feb. 25 to Apr. 1	5832 00	3748 00
Average number of spears harvested per crown for season Feb. 25 to Apr. 1	3 00	1.95
Ratio of number of spears harvested for season Feb. 25 to Apr. 1	1.55	1.00
Total weight of spears harvested from Feb. 25 to Mar. 9 (gms.)	11752.00	5720.00
Average weight of spears harvested per crown from Feb. 25 to Mar. 9 (gms.)	6 02	2.98
Ratio of weight of spears harvested from Feb. 25 to Mar. 9 (gms.)	2.05	1.00
Total weight of spears harvested for season Feb. 25 to Apr. 1 (gms.)	112292.80	82297.60
Average weight of spears harvested per crown for season Feb. 25 to Apr. 1 (gms.)	58.00	43.00
Ratio of weight of spears harvested for season Feb. 25 to Apr. 1 (gms.)	1.34	1.00
Average weight of single spear	19.25	21.90

that the proportion of giant asparagus is much greater from the pistillate plants. This held consistently true throughout their whole population, which was something over 1000 plants.

In a later report on the same project, Tiedjens[8] states that "Staminate plants are higher producing by 25 per cent, and hold up better from year to year.—Pistillate plants produce a greater percentage of 'A' (large) spears."

The data shown in table 6, and derived from experimental plots at the University Farm, Davis, California, bear out the conclusions of previous investigators that there are important differences in earliness of production, total yield, and size of spears between staminate and pistillate plants.

Production of Spears the First Cutting Season.—In 1923 a large number of pistillate and staminate plants were labeled in the nursery. The following winter the crowns were dug and the different sexes planted in separate rows in the permanent bed. The rows were 240 feet long and the distance between rows seven and one-half feet. The crowns were set from one to three feet apart in the different rows. Corresponding staminate and pistillate rows were placed side by side. The harvesting of spears began on February 25, 1925, and continued until April 1. The spears were not cut until they were seven inches or more above the ground. They were cut to a length of nine inches or more in the field. When a row was harvested the spears were trimmed to a uniform length of eight and one-half inches, then counted and weighed. Table 6 gives the number of plants per row, the average weight per crown at time of planting, and the yield of the staminate and pistillate rows for the early part of the harvesting period and also for the entire cutting season. Rows 7 and 8 are Mary Washington, 37, 38, 67 and 68 Washington, and the remainder Palmetto.

The data in table 6 shows that the staminate plants produced more spears and a greater weight of spears during the first cutting

TABLE 7

TOTAL NUMBER AND WEIGHT OF SPEARS HARVESTED EACH CUTTING DAY FROM ALL STAMINATE AND PISTILLATE PLANTS

	Staminate plants (1949)		Pistillate plants (1922)	
	Total number of spears	Total weight of spears gms.	Total number of spears	Total weight of spears gms.
Mar. 2	109	2096	40	970
Mar. 4	171	3416	77	1704
Mar. 6	129	2738	51	1165
Mar. 9	156	3495	77	1802
Mar. 14	239	4985	135	3090
Mar. 16	276	5465	148	3205
Mar. 18	324	6576	223	5390
Mar. 19	480	9915	304	6800
Mar. 21	622	12100	444	9295
Mar. 22	317	6035	183	3960
Mar. 23	511	9716	336	7299
Mar. 24	329	6490	203	4175
Mar. 25	359	6750	279	5920
Mar. 28	328	5595	244	5115
Mar. 27	399	7870	283	6310
Mar. 28	309	5390	195	4140
Mar. 29	371	6765	251	5325
Apr. 1	344	6830	261	6545

season than did the pistillate and that this difference was proportionally greater during the fore part of the cutting season than during the latter part. All staminate rows except No. 51 produced a greater weight of spears than their corresponding pistillate rows. This exception can be easily explained, however, from the small average size of the crowns used in planting row 51, as compared with 52. The spears of the staminate plants were smaller on the average than those from the pistillate plants, but out-yielded the latter by 35 per cent.

The total number and weight of spears cut from all staminate and pistillate plants each harvest day in 1925 are given in table 7.

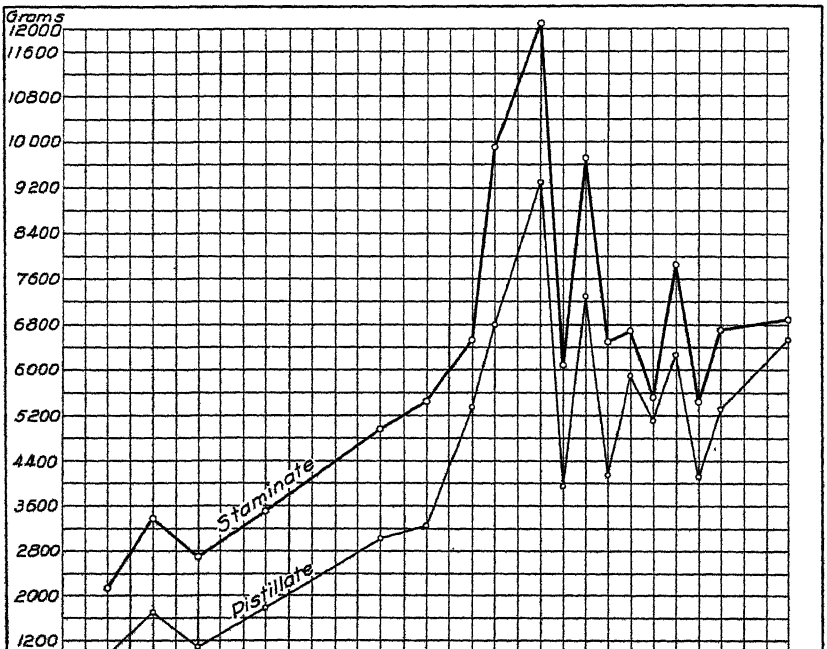


Fig. 6.—Comparison of the total yield of spears from 1949 staminate plants, and 1922 pistillate plants, on different days during first cutting season.

It is seen from the accompanying table and graph (fig. 6) that on no day during the first season of cutting did the number and weight of spears harvested from pistillate plants equal the number and weight of spears from staminate plants. While there are twenty-seven more staminate than pistillate plants, this difference is not significant in such a large population.

SUMMARY

Asparagus officinalis is normally dioecious. All asparagus flowers are apparently potentially hermaphrodite. During floral development there is, except in rare cases, an abortion of one set of sex organs. The following flower forms occur: Strongly pistillate, weakly pistillate, hermaphrodite, weakly staminate, and strongly staminate.

In a large population there are approximately equal numbers of staminate and pistillate plants.

Asparagus has certain secondary sex characters:

(*a*) Staminate plants have a tendency to express their sex earlier in the life of the individual than do pistillate plants.

(*b*) The average height of the first flower-bearing shoot of staminate plants is exceeded by that of the first flower-bearing shoot of pistillate plants.

(*c*) During both the first and second seasons of growth from the transplanting of the crown, staminate plants produce a greater number of stalks on each crown than do pistillate. The difference between staminate and pistillate plants in the green weight of tops is not significant when the berries are included, but if the weight of the green tissue alone is considered, the total weight of the tops of staminate plants is considerably greater than that of pistillate. It appears that the food manufacturing surface of staminate plants exceeds that of the pistillate.

(*d*) During the first harvest season, the staminate plants out yield the pistillate throughout the entire cutting period, but the difference is greatest during the early part of the season.

(*e*) During the first harvest season, the average number and weight of spears from a crown are greater from staminate than from pistillate plants, but the average weight of the individual spears from pistillate plants exceeds that from staminate.

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THE LOCATION AND LONGEVITY IN CALVES OF *BACTERIUM ABORTUM* INGESTED WITH MILK, AND ITS EFFECT ON THE AGGLUTINATION TITRE OF THEIR BLOOD.

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INTRODUCTION AND REVIEW OF THE LITERATURE

The discovery of the elimination of *Bacterium abortum* in the milk of a considerable percentage of cows infected with this organism was made by Schroeder and Cotton[13] in 1911. They found the infection to persist in this location in some cows for a period of years, and this has been confirmed by investigators in various parts of the world. Its presence in the mammary secretion affords an ideal opportunity for abundant ingestion of the organism by the offspring during the first months of its life.

In abortion-infected herds, it is very common to see the disease manifest itself in heifers with their first pregnancy even after premature expulsion of the fetus has practically ceased in the multiparous females owing to the establishment of herd immunity. This naturally suggested that, if the organism could remain viable in the udder of infected cows for a period of five to seven years, or longer, it might remain viable in the bodies of calves during the one or two years from the milk-drinking period until puberty and breeding took place, and be responsible for the abortions in these animals with their first pregnancies. While this hypothesis was reasonable, it was advanced in such a way as to lead many to believe it to be an established fact. Moreover to it was added a second hypothesis, viz., that the only way to prevent infection and subsequent abortion in primiparous animals raised in infected herds was to feed them with pasteurized milk throughout the milk-drinking period of their lives. Were this the true state of affairs regarding the disease, great practical difficulties would stand in the way of controlling this method of infection. A considerable amount of investigational work has been carried out during the last few years to establish the true status of this phase of *Bacterium abortum* infection.

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* C. M. Carpenter took an active part in this work during the first six months, from December, 1922, to June, 1923, and published a report of this early part of it in The Cornell Veterinarian, 19:16–31.

Williams has been the most persistent advocate of this theory, although during recent years he has modified his views. In 1924, in discussing the transmission of permanent genital infection from parent to offspring, he and his co-workers[19] are quoted as follows:

> "It is stated[5] that the bacillus abortus infection acquired by the fetus in utero or by the newborn, does not persist until breeding age and cause injury. This claim has led some to believe that no genital infection may be so transmitted. We make no claim that the *B. abortus* infection is thus transmitted—that is not our theme."

Before the full significance of this question became apparent, Mohler and Traum,[9] in 1911, obtained positive complement fixation and agglutination reactions from a prematurely-born calf which died of scours when ten days old in an infected herd. Experimentally, however, they were unable to produce any noticeable ill effects on two three-day-old calves that were each fed with 90 mils of a *Bacterium abortum* bouillon culture in milk for a period of three days.

McFadyean, Sheather and Minett,[8] in 1913 reported an experiment in which a cow was given *Bacterium abortum* intravenously to see whether the calf would become infected by means of the milk or from contact. The blood of the cow became positive, but the calf remained uniformly negative up to the time of separation from the cow and as long thereafter as it was tested. No attempt was made to demonstrate *Bacterium abortum* in the milk.

In 1916, Schroeder and Cotton[14] demonstrated the high agglutinating power of colostrum from cows with infected udders, followed by a rapid decline in titre as milk took the place of colostrum. Blood of new-born calves suckling dams whose udders were known to be heavily infected with *Bacterium abortum*, although having about the same agglutination titre as that of the dams, still showed a rapidly declining agglutination reaction. They found that *Bacterium abortum* ingested with milk does not seem to penetrate deeply or abundantly into a calf's body. They were able to engender agglutinating proper-

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([5] Schroeder, E. C. Investigations on Bovine Infectious Abortion, Rep. U. S. Live Stock Sanitary Asso., 1921, p. 65.)

ties in the blood of calves by injecting them with abortion organisms, but such injections had to be repeated from time to time, otherwise the agglutinating properties of their blood serum disappeared.

In the same year, Eichhorn and Potter,[4] in field observations on a large dairy herd, noted the history of forty-one animals born in 1911 but kept separate from adult cattle after they could sustain themselves, although having had ample opportunity to acquire the infection by association with infected mothers and the ingestion of infected milk. Nine aborted in 1915 after producing two normal calves each and one, three calves. One aborted twice, having produced one calf, one aborted three times, and one, once, without living offspring. Three aborted in 1914 after having calved normally. They state that if it is a fact that calves acquire the infection shortly after birth and the organism remains latent in the animal's body awaiting pregnancy, it is strange that abortion should not have occurred before the second, third or even the fourth pregnancy.

In 1917, Huddleson[5] reported the feeding of new-born calves with milk that reacted positively to the agglutination test, and the presence of *Bacterium abortum* was further confirmed by guinea pig inoculation. The calves were separated from their dams shortly after birth and fed the infected milk, from a pail, twice daily. The effect of the feeding was studied by means of the agglutination and complement fixation tests upon the blood sera. Eleven calves were fed naturally-infected milk and six were fed non-infected milk, the latter being allowed to suckle their dams for three days before being separated. Non-infected milk consisted of milk that did not show the presence of *Bacterium abortum* agglutinins. One control animal was fed pasteurized, naturally-infected milk and a second was given non-infected milk plus 5 mils of a 48-hour bouillon culture of *Bacterium abortum* with each feeding.

The effect of the feeding was studied by recording the presence of agglutinating or complement fixing substances in the blood of the animals, and the author concludes that such bodies are very rarely demonstrated in the blood of calves as a result of ingesting naturally-infected milk. The data does not exclude the probability that even in the rare cases in which agglutinins did appear, they were the result of ingesting colostrum or milk containing agglutinins. He stated that it would not be logical to assume that his experimental calves acquired the positive reactions as a result of ingesting infected milk since the blood sera of the calves were not tested until after they had received milk.

In a later report, however, published in 1924, covering the significance of *Bacterium abortum* antibodies in the blood of ten heifer calves and one bull, Huddleson and Hasley[6] quite clearly brought out that the ingestion of colostrum is responsible for the positive complement fixation and agglutination tests obtained in most cases. However, in case 1000 A.3, the development of a positive complement fixation test after nursing is unexplained.

In one other case, the authors state: "The blood serium of N.6A was positive to both tests before nursing thus presenting a clear-cut case of what might be termed prenatal infection." The following data are given on this case:

"N.6 A. Born May 14, 1920, one month premature. Very weak. Fetal membranes retained. Dam previously infected by feeding cultures of *Bact. abortus. Bact. abortus* isolated from fetal membranes and uterine exudate at parturition. Milk not examined. Blood of dam positive 1:500 to agglutination test, 0.005 to complement fixation test. Blood of calf positive to both tests before nursing. Nursed dam for three days, then placed on negatively reacting cow for six weeks. Blood remained positive until July 8, 1920."

The positive reaction before nursing was in the first tube only (0.04 mil) and in 0.1 and 0.04 mil to the complement fixation test. When six days old, after nursing the dam three days and then being changed to a negatively reacting cow, its blood was positive with 0.005 mil to both tests.

Prenatal infection of fetuses is well known, but that this infection causes antibodies to appear in their blood has not been established. Reactions in the titre shown by calf N.6 A before nursing are not considered positive in adult animals, and they may have been nonspecific.

Marked discrepancies appeared between the results of the agglutination and complement fixation tests. The reason for this was ascertained during the course of the work and is explained by the use of antigen of too dense a turbidity in the agglutination test. With this we agree, as we have found that the increased turbidity of agglutination test antigen above the ideal standard rapidly decreases the efficiency of the test. The establishment of an ideal standard for the antigen in this test is one of the most important steps to be taken in avoiding discrepancies in its application under various conditions and in different laboratories. We never use an antigen more turbid than that represented by a 3.5 cm. Gates opacimeter reading and one of 5 or 6 centimeters may ultimately prove to be the most acceptable standard.

In 1918, Robinson[12] carried out an experiment with sixteen cows and their calves or fetuses to see whether or not offspring of infected cows showed any production of agglutinins in their blood sera. No positively reacting fetal serum was obtained. One calf, born too weak to stand and suck, showed an agglutination in a dilution of 1 to 25, while its dam was positive in 1 to 500. In two cases the titre of the calf and of the cow serum was the same. In discussing the result of his Experiment No. 3, he states: "In the case of calves born alive and healthy, one may or may not get a weak agglutination, though in a few cases a high one is obtained, but there seems to be no definite ratio between the agglutination of the cow's serum and the calf's."

In the same year, Rettger and White,[11] in a study of infectious abortion in a mixed dairy herd, found that calves became negative to the agglutination and complement fixation tests after the age of two to six months, although shortly after birth they invariably reacted in the same way as the mother. They remained negative until nine to ten months old or the period of sexual development and maturity. After this time whether they were from infected mothers or not, little difference was observed in the development of positive reactions.

Thirty-five daughters of twenty-nine positive cows were listed by the side of forty calves from thirty non-reacting dams over a period of four years (1914 to 1917). Fourteen, or 40 per cent, of the former gave a positive reaction, and seventeen, or 42.5 per cent of the latter, were positive to at least one test. Ten daughters from positive dams aborted, two or them twice, and eleven daughters of negative dams aborted, two of them twice. That the reactions of the dams have no influence on the later history of the calves is clearly brought out in these data.

Seddon[15] reported, in 1919, three cases in calves in which the animals were infected with *Bacterium abortum* without giving any evidence of agglutination reaction. One was a case of a full-term calf born dead from which *Bacterium abortum* was isolated from the stomach contents. A second was a calf expelled alive, at 262 days gestation, from an artificially-infected dam with infected uterus. The third was a calf which, at eighteen days of age, was injected intravenously with 2 mils of living culture emulsion. Thirteen blood tests with blood taken from the third to the seventieth day after the inoculation failed to give a positive reaction. It was then given a second injection at eighty-eight days of age, after which ten blood tests, made from three to fifty-three days after the second injection, all failed to give a positive reaction. Three other calves inocluated in the same manner, when the second injection was given, developed a positive reaction in eighteen days.

In the same year, Dick[3] reported field observations (begun in 1916) on three herds, in all of which the calves received raw milk from the respective herds until weaned.

In herd A, heifers of breeding age were kept with the main herd, and, in 1918, twenty-two, or 44 per cent, aborted.

In herd B, the young stock, instead of having a community pasture, were kept in a separate lot on the same farm, but the segregation was not absolute. In 1918, two of the twelve head, or 16.6 per cent, aborted.

In herd C, the calves were kept on a separate farm until within two weeks of calving time. Three-year records are given for this farm, as follows:

Year	Abortions	Parturitions
1916	1	18
1917	none	23
1918	none	23

Previous to 1916, a large percentage of the heifers on this farm had aborted each year.

In 1920, Simms and Miller[16] reported experiments in which they had fed forty-six heifer calves milk from infected cows known to contain *Bacterium abortum.* All of these calves were negative to the agglutination test before they reached six months of age. After being bred they were handled in such a manner that they were not exposed to infected cows or premises. At the time of the report, twenty-three had terminated their first pregnancies at full term in a normal manner. Agglutination tests of their blood remained negative throughout the respective gestation periods. The writers suggested that there was a possibility of a quiescent, localized infection in such cases, but believed that were this true, the negative heifers might change to positive after pregnancy had taken place. No evidence of such an occurrence was obtained.

Until this time a thorough understanding of the relationship of colostrum to the presence of antibodies in the blood of newborn calves was lacking. This was clearly brought to the attention of investigators by the work of Little and Orcutt[7] in 1921. For several years previously, these workers had tested the blood sera and transudates of fetuses from time to time to determine the relation between concentration of abortion agglutinins in the blood sera of mother and fetus. The results showed that even when the serum of the mother had a high agglutination content, little or none was found in the fetal blood.

In experiments covering twenty cows and nineteen calves, the fact was established that even when the blood and colostrum of the dam had a relatively high agglutination content, the blood of the calf, with a single exception, was free. When colostrum was withheld and milk of a lower or negative agglutination titre substituted, agglutinins failed to appear in the calves' blood after several days.

The antibodies in a calf's blood are absorbed from the digestive tract, the rate of absorption being fairly rapid. In slightly over an hour after the feeding of colostrum, they began to appear and had nearly reached their maximum concentration five hours after feeding.

These experiments made it evident that agglutinins for *Bacterium abortum* found in the blood serum of new-born calves are obtained from the mother through the colostrum. Calves at birth, unfed, are without agglutinins.

In 1922, Duebler[2] reported on a group of twenty heifers raised several years previously under identical conditions until two months before breeding. Ten were then placed in barns with the cow herd and the other ten in a barn for young stock only, having no connection with the breeding herd. All were bred. Seventy per cent of the heifers in the cow barn aborted, and 100 per cent of the other group calved normally. From then on to the time of reporting, all heifers on the farm were bred and kept in the heifer barn, and, with an average of fifteen to twenty heifers calving yearly, none had aborted.

Quinlan[10] reported, in 1922, a series of experiments in South Africa in which he used five groups of calves, totalling forty-one head, with one control animal. The infected milk given the calves was from cows whose blood and milk samples gave positive agglutination tests. In some cases, the presence of the organism was actually demonstrated by guinea pig inoculations.

In these experiments the author attempted to determine by the agglutination titre of the blood serum of the calves the effect of the feeding of infected milk. The titre was studied in some cases until the calves reached maturity.

In Group A of eleven head separated from their infected dams and artificially fed infected milk and Group B of four head, born to infected dams and allowed to suckle them, antibodies were found in the blood of five.

In Group C of eighteen head from non-infected dams fed infected milk and in Group D of six head from non-infected dams, but fostered by infected dams, specific antibodies were found in the blood in only three cases.

The author makes the statement that "apparently the infection which results before birth through the infected mother is to be considered in far greater measure the cause of the appearance of the antibodies in the calf's blood than the ingestion of infected milk."

The author cites an interesting case, Friesland Bull 89, one of the two head of Group E. This calf was born from an infected cow and was fed its mother's milk for three days. The milk of this cow had never given a positive reaction to the agglutination test. The calf, when born and when removed from his mother, showed a slight agglutination with .025 mil of serum, but after having been fed non-infected milk for one month and eighteen days, showed an agglutination titre of .001 mil. This, the author states, "appears to be a case of temporary active infection and moreover the infection seems to have been of intrauterine origin."

Despite the finding of agglutination reactions in the early days of the lives of the experimental calves, the author found that the agglutination titre tended to fall and disappear altogether after twelve to fifteen weeks even though infected milk was still being fed regularly.

When antibodies were present at the time feeding infected milk was stopped, they disappeared shortly afterwards. In some cases, calves were fed exclusively with infected milk up to the ninth month, without provoking the appearance of antibodies in their blood. It was observed that the calves remained in excellent condition despite the ingestion of the abortion organisms. The results also prove that calves fed infected milk do not become chronic carriers of the disease. The author was able to follow up the history of nearly all the female calves used in the experiment and in no case found any evidence that an animal become a carrier.

The value of a proper understanding of the agglutination reactions in calves is great, and, we cannot but feel after considering his work that, at the time of preparing his report, the author was not familiar with the very important role played by positive colostrum in this respect as disclosed by the work of Little and Orcutt.[7]

In 1924, Barger and Hayes[1] reported experiments in which *Bacterium abortum* was given in the milk to three groups of two calves each to ascertain whether it could be recovered from the feces. Both naturally and artificially infected milk were used. The organism was successfully recovered from the feces of the calves and it was also found in the lymph glands of the head for seventeen and nineteen days respectively in Groups B and C after the discontinuance of the infected milk. Agglutinins did not appear in the blood sera of the calves fed with infected milk for periods ranging from fifteen to twenty-one days.

PLAN OF THE EXPERIMENTS

In our study of this problem, thirty-nine calves, including both sexes, were used. They were all born to our experiment cows and were kept under our direct supervision and control from birth until they died, were killed, or terminated their first gestation period. Some of them received colostrum and from others it was withheld.

The infected milk with which these calves were fed contained *Bacterium abortum,* in some instances, when drawn from the udder, but, in all cases, a definite quantity of a known pathogenic strain of the organism was added to the milk before each feeding.

There were some deaths in the calves a few days after they were started on the infected milk. The remainder were slaughtered from seven days to twenty-four months afterwards, the period between the time infected milk was withdrawn and the date of death varying from seven days to eighteen months.

When the calves were slaughtered, cultures were made and guinea pigs inoculated from various body tissues to test for the presence of *Bacterium abortum.*

All calves living longer than six months were weaned at that age. Eleven head were allowed to reach sexual maturity, breed and complete one gestation period, when their colostrum and placentae were examined for the presence of the organism. These animals were bred at about fifteen months of age. A shorter period of time than ordinarily obtains in practice, therefore, elapsed between the last exposure to *Bacterium abortum* infection and the establishment of pregnancy.

Blood samples were collected and agglutination tests made at frequent intervals during the lives of the calves.

PREPARATION OF THE SUSPENSION OF *BACTERIUM ABORTUM* ADDED TO THE MILK

Throughout the work, the strain of *Bacterium abortum* used was our Laboratory No. 80, originally obtained from K. F. Meyer. This was an especially virulent strain of the organism.

From December 5, 1922, to January 25, 1923, each calf that was being fed received daily the growth on one glycerin glucose agar slant culture washed off with about 20 mils of physiological saline solution. One-half of this was added to the morning and the remainder to the evening feed of milk. After January 25, on account of the increased number of calves, the organism was grown on the same medium in Blake bottles. The growth was washed off and the the suspension standardized to 1.5 cm. Gates opacimeter reading. A suspension of this density of the *Bacterium abortum* organism from a 48 to 72-hour culture will show an average of five to six billion organisms per mil by the plate culture method. A half-ounce vial of this suspension was added to the milk for each calf night and morning.

From January 25, 1923, to January 23, 1924, when the last dose of the organism was given to calf 1780, sixty-one batches of the suspension were made up. One guinea pig was inoculated intraperitoneally with 1 mil from each batch except the first, to test its virulency. Fifty-nine of the sixty guinea pigs developed abortion disease. Twenty-six died from the infection and thirty-three were killed approximately six weeks after the date of inoculation. One guinea pig died from intercurrent disease a few days after inoculation.

RESULT OF THE EXAMINATION FOR THE PRESENCE OF *BACTERIUM ABORTUM* IN THE TISSUES OF CALVES RECEIVING THE ORGANISM IN MILK FOR VARYING PERIODS OF TIME

When the calves were slaughtered, two cultures were made, on glycerin glucose agar and cooked blood agar, respectively, from the following tissues with the exceptions that appear in the table: atlantal, submaxillary, posterior pharyngeal, mediastinal, bronchial, gastric, hepatic, mesenteric, superficial inguinal or supramammary, pelvic, prescapular and precrural lymph glands, thymus gland, liver, spleen and stomach and intestinal contents.

From ten to twelve guinea pigs were injected with the tissues of each calf, parts of several glands being inoculated into a single guinea pig in some cases.

Table 1 gives the result of this examination.

In table 1, calf 1732 did not receive infected milk until it was seventeen days old, D–IV calf until it was three days old and calf 1763 until it was forty days old. The last did not receive infected milk during the first six weeks of its life when it was running in pasture with its non-infected dam. Calf 1745 did not receive the organism during the last three days of its life because it was given little milk on account of severe diarrhea. When cultures are marked negative, it signifies that *Bacterium abortum* was not recovered. The cultures were not always sterile. In some cases, where the calves died of scours *B. coli* overgrew the tubes.

In addition to the tissues listed in the table, cultures were also made in fifteen cases from the testicle, in nine from the uterus, in eight from the kidney, in eight from the epididymis, in six from the vagina, in four from the heart, in three from the gall bladder and in two from the lung. All resulted negatively, except one culture from the epididymis of calf 1753, which developed *Bacterium abortum*.

Two of the animals were pregnant when killed. No. 1778 was about six weeks and No. 1775, five months along in gestation. The fetus and membranes from No. 1778 were triturated in a mortar with saline solution and injected into one guinea pig. From the much further developed fetus of No. 1775, three guinea pigs were injected from the maternal and fetal cotyledons; lung, liver and spleen extract, and stomach and intestinal contents, respectively. All resulted negatively.

Two cultures were also made from the lung, liver, spleen, and stomach contents, respectively of the fetus from No. 1775 and remained sterile except for a mold on one tube and a few large cocci on another.

Twenty eight calves died or were killed and their tissues examined in the above manner, the remaining eleven being allowed to complete one gestation period.

The table shows that *Bacterium abortum* ingested in milk passes through the walls of the digestive tract and gains access to various lymph glands and other organs, particularly the spleen. In no case was the organism found in the thymus gland.

The lymph glands about the head became infected with great regularity, showing that *Bacterium abortum* is taken up from the mouth and throat. It is also present, as would be expected, in the lymph glands along the digestive tract. However, its presence in the bronchial and mediastinal glands was quite commonly observed. In the more remote body glands, it can rarely be demonstrated. The findings show that the organism can undoubtedly pass through the intact mucous membrane of the digestive tract, be taken up by the lymphatic glands and, in some cases, must gain access to the blood stream from which it may occasionally be deposited in almost any tissue of the body.

Whether the calf receives colostrum or not seems to have no bearing on the invasion of the organism. Despite its constant penetration and possibilities for quite general distribution, there does not seem to exist in either the male or female calf any location that furnishes a favorable place for more than temporary existence in the absence of a constant supply of fresh infection.

A few weeks after infected milk is withdrawn, the organism is rarely demonstrated and undoubtedly in the great majority of cases under natural conditions the bodies of calves are free from the organism in from six to eight weeks after the cessation of infection.

Among our experiment animals where very heavy dosing had occurred, No. 1758 was found to contain the organism in the atlantal gland at the time of slaughter, seven weeks after infection had ceased, and it was recovered culturally from the submaxillary lymph gland of calf 1772 when killed, eleven weeks after the last infection. Six of the animals, including the two mentioned above that became pregnant, were examined after living longer following the removal from sources of infection, and all were negative.

AGGLUTINATION REACTIONS OF THE BLOOD OF CALVES TO *BACTERIUM ABORTUM* ANTIGEN

The effect of the ingestion of the milk or colostrum and milk containing *Bacterium abortum* on the agglutination reactions of the calves has been carefully observed by taking blood samples at regular intervals throughout the two and one-half years covered by the experiment. This is given in table 2.

It will be observed by reference to this table that, despite the excessive quantity of virulent *Bacterium abortum* being ingested daily by the calves, their agglutination tests were generally negative.

Colostrum was withheld from some of the calves with two separate objects in view. The first was to study any difference in the invasive ability of the organism and the agglutination titre of the blood as compared with that of the dams. The second was to ascertain the effect of the presence or absence of colostrum ingestion on the development of gastro-intestinal disturbances.

When colostrum was withheld from calves, an attendant was present at time of birth. In case any doubt existed as to the calf possibly having received colostrum, it was allowed to remain with the dam and was placed in the group that received colostrum.

During the first ten days of their lives, calves in the no-colostrum group were fed milk from cows with negative agglutination titre and that had been fresh for a period of at least seventeen days. Only No. 1736 received milk from cows this early in lactation. It was born on December 20, 1922, and received milk that night from cows 2312 and 26 which had both freshened on December 3, 1922, and this was the only milk available. It was recognized that in case gastro-intestinal disturbances were going to occur, they would develop in the great majority of cases during this period. After the ten days had elapsed, their feeding could not be watched so closely from this standpoint on account of the number of calves being handled and the availability of the desired kind of milk. Also, our chief concern at this time consisted in seeing that *Bacterium abortum* was only ingested by the calves that we desired to have it and not by those that were being kept for periods of time after its withdrawal before slaughter and examination.

Some of the calves received milk indefinitely from cows fresh thirty days or longer, but, in these cases, the possibility that they received milk from the same bucket that had previously contained positive colostrum or milk without rinsing was quite likely to have occurred.

Sixteen calves received no colostrum. The first blood sample of all of these calves was negative, except that of No. 1751, which was positive in only the .02 mil tube and negative in .04 mil, .01 mil and .005 mil on the day after its birth. This reaction is paradoxical: the blood of the dam was negative—our laboratory records show it was not rechecked, and faulty technique may have been responsible. Of the sixteen dams of these calves, nine showed a positive agglutination reaction in a titre of .04 mil to .01 mil, the remaining seven were negative.

In the case of the twenty-three calves which received colostrum, nearly all were from negative dams. Only Nos. 1759 and 1749 deserve mention. The blood of these calves showed absence of agglutinins when tested five and seven days respectively after birth from cows that showed a positive blood agglutination in a titre of .02 mil and .01 mil respectively. One of these, No. 1759, was dead of scours at the time the blood was taken. Unfortunately we did not test the agglutination titre of the colostrum of these cows and it may be that it was very low or negative in both cases. That ingestion of positive colostrum by calves will quickly result in agglutinins being present in their blood is very easily demonstrated.

In studying table 2, it will be observed that in some cases the titre of the blood of the calves changed from negative at one test to positive with .005 mil of serum at the next. This may have been due to the fact that colostrum or milk having a high agglutination titre was fed to these calves in the interim between the tests. However, the probability of an occasional temporary active production of agglutinins within the calf as a result of the ingestion of large quantities of *Bacterium abortum* must be considered.

In this connection we would mention particularly Calf 1736. This animal was born December 20, 1922, to Dam 2404, showing a suspicious blood agglutination test but in which *Bacterium abortum* could not be demonstrated at time of parturition. It was immediately removed from its dam without getting colostrum and fed with milk from two negative cows, plus the *Bacterium abortum* suspension, until March 23, when it was given milk from a positive cow. On December 27, 1922, when seven days old, its blood was entirely negative. On January 8, 1923, when nineteen days old, its blood was very positive with .01 mil and partially so with .005 mil. On January 17, 1923, when twenty-eight days old, it had again become entirely negative. This case may be taken as evidence that *Bacterium abortum* ingested with milk stimulated the very temporary production of antibodies in the blood. Even if it is the proper explanation of the rise in agglutination titre, of the other cases in table 2 it is of irregular occurrence and its duration covers a short period of time.

The possibility of intra-uterine infection having a bearing on the presence of agglutinins in the early life of calves has attracted our attention principally as a result of the following statement by Little and Orcutt[7] in their work in demonstrating positive colostrum to be the carrier of agglutinins to new-born calves: "The problem of the production of agglutinins by the fetus in whose tissues *B. abortus* has multiplied and which is subsequently expelled prematurely is not touched by these observations."

We have run agglutination tests on the blood of twenty-one fetuses in the bodies of which we have demonstrated *Bacterium abortum* by cultural methods and guinea pig inoculations. In every instance these tests were entirely negative in the four dilutions of .04 mil, .02 mil, .01 mil and .005 mil, respectively. The result of this work is tabulated in table 3.

We are of the opinion that this series of tests corroborates the work of others and demonstrates that the pentration of the tissues of a fetus by *Bacterium abortum* does not result in the production of agglutinins by the fetus in utero.

TABLE 3.—SHOWING NEGATIVE BLOOD REACTIONS OF FETUSES INFECTED WITH *BACTERIUM ABORTUM* IN UTERO

No. of fetus	Period of gestation	Agglutination test of blood	Tissues from which *Bacterium abortum* was isolated							
			Lung		Liver		Spleen		Stomach and intestinal contents	
			Culture	G. P.	Culture	G. P.	Culture	G. P.	Culture	G. P.
7	9 months.....	----	–	+	–		–		–	+
10	7 months. . .	----	+	+					–	+
11	7 months.......	----	+	+	–				+	+
12	7 months........	----	+	–					+	+
13	8 months......	----	+	+					+	+
14	7 months ...	----	+	+					–	+
15	8 months....	----	+	+					+	+
18	7 months.......	----	+	+					+	+
20	7 months	----	+	+					+	+
25	8 months.....	----	+	+					+	+
35	8 months.......	----	+	+					–	–
37	9 months......	----	+	+					+	+
41	6 months.......	----	–	+	–	+	+	+	+	+
46	8 months......	----	+	+	–	+	+	+	+	+
49	8 months......	----	+		–		–		+	+
50	9 months........	----	+	+	–	+	–	+	+	+
53	8 months.......	----	–	+	–	+	–	+	–	–
57	7 months......	----		+		+		+		+
A.E.IV	5½ months.. .	----	–	+	+	+	+	+	+	+
A.E. V	6½ months....	----	+	+	+	+	–	+	+	+
A.E.VI	6 months......	----	–	+	–	+	+	+	+	+

EFFECT OF THE SUBCUTANEOUS INJECTION OF LIVE AND DEAD SUSPENSIONS OF *BACTERIUM ABORTUM* ON THE AGGLUTINATION TITRE OF THE BLOOD OF CALVES

On account of the failure of *Bacterium abortum* infection of fetuses in utero and also the failure of the ingestion of excessive quantities of the organism in milk from birth to six months of age to produce agglutinins in the blood of calves, we carried on a few experiments to see with what regularity subcutaneous injection of live and dead organisms would cause the development of these bodies in the blood of the injected animal.

The first test involved only one calf, born October 4, 1924, to a negative dam. This calf was injected subcutaneously on the side of the neck with 10 mils of a suspension of *Bacterium abortum*, having a .9 cm. Gates opacimeter reading on October 30 and again on November 20, 1924. Blood was drawn from the calf on October 29, November 5, 10, 17 and 25 and December 2, in all of which it was completely negative. On November 25, a local swelling 4 inches in diameter was present where the injection of the organism had been made November 20, 1924.

On December 22, 1924, blood was taken from the calf again and showed a positive reaction in the first tube titre .04 mil. In looking for an explanation of this reaction, it was found that the attendant had milked into his pail a positive cow, which calved December 1, 1922, had emptied the milk out and then, without rinsing the pail, had proceeded to milk the negative cow supplying the calf. The blood of the positive cow had a high agglutination reaction and her colostrum was positive at a titre of .0015 mil. This contaminated pail had been used several times between December 2, when the last negative blood sample was taken from the calf, and December 22, when the blood of the calf showed a positive reaction in the first tube. While the low titre agglutination might have been the beginning of a reaction from the injected organisms the experiment was terminated.

This calf had received two injections of live organisms and a period of thirty-three days had elapsed from the first one without showing any reaction, although it is quite well established in adult cows that, with subcutaneous injection, seven to ten days are sufficient to produce a well marked, positive reaction in a titre of .01 mil or higher.

On March 30, 1924, four more negative calves were selected to receive a killed suspension of the *Bacterium abortum* organism, of the same dose and density as that used with the first calf. These calves were all injected March 30, April 14 and April 28. The following blood reactions were obtained:

No.	Date of birth	Feb. 27, 1925	Mar. 30, 1925	Apr. 15, 1925	Apr. 28, 1925	May 11, 1925
1831	Jan. 12, 1925	− − − −	− − − −	ǂǂǂ∓	ǂǂǂǂ	ǂǂǂǂ
1833	Jan. 5, 1925	− − − −	− − − −	ǂǂ±∓	ǂǂǂǂ	ǂǂǂǂ
1835	Jan. 18, 1925	− − − −	− − − −	− − − −	ǂǂǂǂ	ǂǂǂǂ
1838	Feb. 5, 1925	− − − −	− − − −	ǂǂǂ−	ǂǂǂǂ	ǂǂǂǂ

With the development of the very positive reaction in all four of the animals, this experiment was terminated.

On May 11, 1925, another series of four calves was selected to receive live organisms of the same dose and density as used in the first experiment. Three of them were negative and one was positive as a result of nursing a positive dam. This calf was kept in the group purposely. Each of these calves was injected subcutaneously with the organisms on May 11, May 27 and June 14. The following blood reactions were obtained:

No.	Date of birth	Apr. 27, 1925	May 11, 1925	May 27, 1925	June 14, 1925	June 30, 1925
1842	Apr. 21, 1925		−−−−	−−−−	ǂ++∓	ǂǂ+∓
1843	Apr. 11, 1925	−−−−	−−−−	−−−−	ǂǂǂǂ	ǂǂǂǂ
1844	Apr. 9, 1925	−−±ǂ	∓ǂǂ+	+±±−	ǂ+++	+++−
1845	Feb. 27, 1925	−−−−	−−−−	−−−−	ǂǂǂ+	ǂǂǂǂ

It would, therefore, seem that either live or dead *Bacterium abortum* organisms injected subcutaneously will result in the development of specific agglutinins in the blood of the calf. They are somewhat slower in appearance than with adult animals.

EFFECT ON THE MORTALITY AND MORBIDITY OF CALVES OF FEEDING AS COMPARED TO THE WITHHOLDING OF COLOSTRUM

The work of Smith and Little[17] on the importance of colostrum to the new-born calf clearly demonstrates the value of this secretion in preserving its health. We were desirous of ascertaining if all calves in our experiment receiving colostrum would remain healthy and also if it were at all practical to raise calves from which it was completely withheld during the early period of their lives.

In addition to the thirty-nine calves listed in table 1, ten others were placed in the experiment, but died at such times that they were not recorded in the *Bacterium abortum* studies. There was thus a total of forty-nine head of calves used. Twenty-seven of these received colostrum and twenty-two did not. We observed equal precautions in the feeding of both groups to prevent digestive disturbance and changed the feeding procedure in various ways in an attempt to remedy any abnormal conditions that developed.

The calves were started on small quantities of milk, diluted with water in some cases, and the amount fed gradually increased. During the first few days, the feed was usually given three time a day. In cases where gastro-intestinal disturbances developed, milk was entirely withdrawn for as long as twenty-four hours and barley water substituted. The actual feeding records of calf 1777 in the colostrum group and 1737 in the no-colostrum group are given below as typical.

Calf 1777, born June 29, 1923. Received colostrum from dam June 29 and 30, and morning of July 1.

Date	Morning and Evening (each)	Noon
July 1	1 quart milk (evening only)	
July 2	1 quart milk	1 quart milk
July 3	1½ quarts milk	1 quart milk
July 4 to 7, inc.	2 quarts milk	1 quart milk
July 8	Showed scours 1 quart barley water	1 quart barley water
July 9	1½ quarts milk	1 quart milk
July 10 and 11	2 quarts milk	1 quart milk
July 12 and 13	2½ quarts milk	
July 14 to 19, inc.	3 quarts milk	
July 20 to 25, inc.	3½ quarts milk	
July 26 to Aug. 19, inc.	4 quarts milk	
Aug. 20	Showed scours 2 quarts milk (evening only)	
August 21	2 quarts milk (morning) 4 quarts milk (evening)	
Aug. 22 to Nov. 30, inc.	4 quarts milk	
December 1 to 28, inc.	Gradually decreasing amount of milk until weaned	
December 29	Weaned	

Calf 1737, born December 31, 1922. Immediately removed from dam without getting colostrum.

Date	Morning and Evening (each)	Noon
December 31	¾ quart milk and ¼ quart water (evening only)	
January 1	½ quart milk ¼ quart water	
January 2	Started to scour ½ quart milk } (morning) ¼ pint water } ½ quart barley water (evening)	½ quart milk ½ quart water
January 3	½ quart milk ½ quart water	½ quart milk ½ quart water
January 4	¾ quart milk ½ quart water	¾ quart milk ½ quart water
January 5 to 12	1 quart milk ½ quart water Evening of January 11 calf would not eat, but apparently not sick.	1 quart milk ½ quart water
Jan. 13 to Feb. 10, inc.	1 quart milk ½ quart water	1 quart milk ½ quart water
February 11 to 19, inc.	1½ quarts milk	1½ quarts milk
February 20 to 22, inc.	3 quarts milk	
Feb. 23 to March 4	4 quarts milk	
March 4	Killed.	

Three of the calves receiving colostrum, Nos. 1759, 1761 and 1764, died during the first few days of life from scours. Four others, Nos. 1749, 1746, 1777 and 1773, showed some evidence of scours but it did not terminate fatally. Twenty remained normal.

Eight of the calves which did not receive colostrum died of scours, Nos. 1745, 1747, 1757, 1740, 1743, 1756, 1760 and 1768. Six others, Nos. 1732, 1737, 1741, 1744, 1754 and 1742, showed evidence of scours but recovered. No. 1742 died later from poisoning. The remaining eight developed no gastro-intestinal disturbances.

This tabulation gives the feeding of colostrum a decided advantage in preserving the health of the calf in early life. However, it also shows that this is not the only essential factor in all cases in preventing intestinal disturbances as evidenced by the deaths in calves which received it. This same observation is made in the practical raising of calves in dairy ranches in this state. We have seen one serious outbreak of scours in calves where it was the practice to leave the calf with its dam for a period of several days to one week or longer.

RESULT OF EXAMINATION OF PLACENTAE AND COLOSTRUM FOR *BACTERIUM ABORTUM* IN CASES WHICH CALVED

Ten of the eleven heifers that were allowed to reach maturity and breed carried their calves to term. The result of the examinations of their placentae and colostrum is given in table 4.

These heifers became pregnant at from thirteen to nineteen months of age. All were bred by bulls 411 and 412, which had been used in our original series of abortion experiments, and, therefore, during 1922 and 1923 had been associated with some aborting cows and cows that had been vaccinated with living *Bacterium abortum.* Neither bull became infected by this association and their blood gave negative reactions to the agglutination test. Five services were required to get No. 1736 pregnant. In the first four of these, bull 412 was used and, at the time, he was neither a very active nor sure breeder. The first breeding with bull 411 was successful in getting the animal pregnant. Three services were required to establish pregnancy in No. 1751, the first two being with bull 412, and two for No. 1767, both with bull 411, the remainder conceiving with the first service.

Eight of the ten heifers calved normally. No. 1738 started to calve during the day of November 16, 1924, but was not seen by the attendant until 5 p. m., when she was found lying down with the posterior end of the calf protruding from the vagina. As the attendant rode up to her on a saddle horse, she got up and parturition was

TABLE 4.—BREEDING AND CALVING DATES OF THE ANIMALS ALLOWED TO REACH MATURITY AND RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF *BACTERIUM ABORTUM*

Ear tag No.	Date of birth	Breeding date	Approximate age when conception occurred	Calving date	Guinea pigs injected placenta	Guinea pigs injected colostrum	Guinea pigs killed	Blood reaction of guinea pigs	Post mortem of guinea pigs	Cultures from spleens of guinea pigs	Placenta
1738	Jan. 17, 1923	Feb. 10, 1924	13 months	Nov. 16, 1924	4153–54	4155–56	Jan. 6, 1925	—	—	—	Manually removed Not adherent.
1744	Jan. 29, 1923	May 13, 1924	15½ months	Feb. 18, 1925	4359–60	4361–62	Apr. 3, 1925	—	—	—	Expelled normally.
1755	Mar. 6, 1923	June —, 1924	14 months	Mar. 9, 1925	4392–93	4394–95	Apr. 20, 1925	—	—	—	Expelled normally.
1748	Feb. 7, 1923	June 17, 1924	15½ months	Mar. 21, 1925	4433–34	4431–32	4433–34 died. Others killed May 4, 1925	4433–34 not posted. Others —	4433–34 not posted. Others —	4433–34 not posted. Others —	Expelled normally.
1736	Dec. 20, 1922	Apr. 30, May 21, June 13, July 4 and July 25, 1924.	19 months	May 1, 1925	4534–35	4536–37	June 11, 1925	—	—	—	Expelled normally.
1778	May 23, 1923	July 31, 1924	14 months	May 3, 1925	4538–39	4540–41	June 15, 1925	—	—	—	Expelled normally.
1754	Mar. 4, 1923	July 31, 1924	17 months	May 6, 1925	4552–53	4550–51	4553 died. Others killed June 18, 1925	4553 not posted. Others —	4553 not posted. Others —	4553 not posted. Others —	Expelled normally.
1769	May 2, 1923	Aug. 19, 1924	15½ months	May 23, 1925	4574–75	4572–73	July 8, 1925	—	—	—	Expelled normally.
1767	Apr. 16, 1923	July 25 and Aug. 27, 1924.	16½ months	June 1, 1925	4588–89	4586–87	July 14, 1925	—	—	—	Expelled normally.
1751	Feb. 13, 1923	May 29, July 21, Sept. —, 1924.	19 months	July 1, 1925	4665–66	4663–64	Aug. 13, 1925	—	—	—	Expelled normally.

completed, but the calf was dead. The heifer was quite thin and we think parturition had been slow, the posterior presentation resulting in the calf being smothered during birth.

No. 1748 developed a marked case of dystocia, and embryotomy had to be performed to extract the fetus. She came in labor about 6:30 p.m. March 20, 1925. At midnight, the attendant decided the calf could not be expelled on account of its abnormal presentation. Early the following morning, an examination disclosed the head bent down between the front legs and held by the brim of the pelvis. On account of the small size of the animal, it was necessary to remove both front legs before the head could be elevated and the calf extracted. The placenta came away with the calf. The cow developed metritis and died March 24, 1925.

Laboratory examinations of the dead calves born to heifers 1738 and 1748, respectively:

Calf of heifer 1738:
  Brought to laboratory November 17, 1924.
  Fully developed.
  Externally normal.
  Post-mortem examination, normal.
  Cultures made on cooked blood agar, one tube, and glycerin agar, one tube, from each of the following organs:
    Lung. No growth visible.
    Liver. No growth visible.
    Spleen. No growth visible.
    Stomach contents. No growth visible.
    Intestinal contents. No growth visible.
  Guinea pig 4151 was inoculated with lung, liver and spleen extract, and guinea pig 4152 with stomach and intestinal contents. Both were killed January 6, 1925, and were normal. Their blood serum was negative to the agglutination test and cultures made from their spleens remained sterile.

Calf of heifer 1748:
  Brought to laboratory March 21, 1925.
  Externally normal except for fact its two front legs had been removed.
  Post-morten examination, normal.
  Cultures were made on cooked blood agar, one tube, and glycerin agar, one tube, from each of the following tissues:
    Lung. Bact. coli.
    Liver. Bact. coli.
    Spleen. No visible growth. Suspicious Gram negative bodies in smear from this tube. Tissue suspension injected into guinea pig 4525 April 17, 1925. Killed June 3, 1925. Normal. Blood, negative. Spleen culture, sterile.
    Stomach contents. Bact. coli.
    Intestinal contents. Bact. coli.

Guinea pig 4435 was inoculated with stomach and intestinal contents and guinea pig 4436 with lung, liver and spleen extract. Guinea pig 4436 died the following day but no post-mortem was made. Guinea pig 4435 was killed May 4, 1925, and was normal. Its blood serum was negative and a cooked blood agar culture from its spleen was negative.

The guinea pigs inoculated with the placenta and colostrum from all of the ten animals were negative for *Bacterium abortum* (see table 4).

ABORTION OF NON-SPECIFIC CAUSE IN ONE ANIMAL

The eleventh animal, No. 1762, of the group that was to complete one gestation period, aborted in the seventh month of pregnancy. This heifer was bred July 1, 1924, and conceived. On January 22, 1925, she was observed in the pasture gaunt and showing evidence of udder development. Suspecting abortion, the attendant searched the pasture and found the fetus and placenta. With this animal at the time were all the other heifers of this group, except No. 1738, which had calved in November and been removed. This heifer was then taken from the pasture. Colostrum was collected from her udder and, together with the fetus and membranes, was brought to the laboratory.

The fetus was normal, but the placenta was very dry and appeared to be shrivelled. Two cultures were made on glucose glycerin agar from each of the following tissues of the fetus:

Heart, liver, lung, spleen,
stomach contents, meconium rectum.

All of these cultures remained sterile and were discarded after three weeks' incubation.

The following guinea pigs were injected intraperitoneally with material from the case:

No. 4291—Lung, liver and spleen of fetus.
No. 4292—Stomach contents and meconium of fetus.
No. 4293—Placenta.
No. 4294—Colostrum.

No. 4293 was dead the second day after inoculation with generalized peritonitis. Cultures made from the viscera developed Bact. coli.

The remaining three guinea pigs were killed March 4, 1925, and were normal throughout. Their blood was negative to the agglutination test and cultures made from their spleens remained sterile.

The blood of this heifer had been negative to the agglutination test as shown in table 2. This case has previously been reported by Traum and Hart[18] as a case of abortion without demonstrable cause.

CONCLUSIONS

1. This series of experiments demonstrates that *Bacterium abortum* ingested by calves with milk find their way to the lymph glands along the alimentary canal, particularly those about the head, and, in some cases, gain access to the blood stream as evidenced by their frequent recovery from the spleen.

2. These organisms do not remain permanently located in the body tissues of the calf. A few weeks after infection ceases, the organisms are no longer found.

3. The longest period in which they were found after infection ceased was seven weeks in one case in the atlantal lymph gland and eleven weeks in another case in the submaxillary lymph gland.

4. No difference between male and female calves was observed in this connection. The feeding or withholding of colostrum also had no effect on the result.

5. Although the period between the time infected milk was withdrawn and pregnancy was established in the animals that were allowed to reach maturity (seven to thirteen months) was much shorter than normally occurs in dairy farm practice, none of them showed any evidence of *Bacterium abortum* infection.

6. The ingestion by calves of large quantities of virulent *Bacterium abortum* organisms in milk, followed by their gaining access to certain lymph glands and other body tissues, including the blood stream, does not with occasional exceptions result in the production of agglutinins in the blood of these animals.

7. The testing of the blood of calves up to the time they are from nine to twelve months of age is, therefore, of no value in herds where the disease is being controlled by the agglutination test and isolation of reactors.

8. Greater morbidity and mortality were experienced among the calves that did not receive colostrum.

9. Nevertheless, we were able to raise calves without colostrum and its feeding did not, in all cases, stop the development of serious and even fatal gastro-intestinal disturbances.

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PHYSIOLOGICAL ASPECTS OF SOIL SOLUTION INVESTIGATIONS

BY

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INTRODUCTION

In investigations of the chemical system constituted by the soil and the plant, it is unavoidable, in the majority of cases, that the research should be directed into some specialized phase of soil chemistry, or of plant physiology. Little opportunity is afforded to make direct comparisons between soil conditions and the conditions of artificial cultures. For the past ten years, however, the California Experiment Station has conducted various soil and plant investigations which have made possible such comparisons. This has brought up for discussion numerous questions pertaining to the physiological aspects of soil solution investigations, and perhaps it is worth while to pause for a short time, in the course of detailed study of experiments, to take a general survey of several important physiological phases of the soil-plant system.

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* This discussion is based on papers submitted to the International Congress of Soil Science (Pédologie), Rome, May, 1924, and to the Western Society of Plant Nutrition, Stanford University, June, 1924, and Portland, June, 1925. It is intended simply as a critical statement of certain soil solution problems as they have presented themselves during investigations conducted in California; and its limitations preclude any but incidental references to the literature on the subject. In its preparation, discussions with my colleagues in California and with investigators in other institutions have been very helpful. Of especial value have been discussions of soil solution questions with Professor J. S. Burd and Mr. J. C. Martin, and of physiological problems with Professors C. B. Lipman, A. R. Davis and W. F. Gericke.

PHYSIOLOGICAL RELATIONS OF SOIL SOLUTION TO SOIL ORGANISMS

Before proceeding with the main discussion, which concerns the higher plants, it seems indispensable to insert a brief statement regarding the physiological relations existing between the soil solution and the microörganisms of the soil. The statement that an essential condition of fertility in soils is the development of desirable microorganisms has been made countless times and in many forms. Yet this point may require new consideration in connection with current researches on the soil solution. The data obtained on water extracts of soils and the more striking and definite information made available by Burd and Martin[4] through their studies on soils with the use of a modified Parker displacement method, illustrate the fact that normally a soil solution is, in large measure, a biologically controlled system; that is to say, nearly all the anion (NO_3, SO_4, HCO_3) content of such a solution is of biological origin and equivalent quantities of cations must enter into solution along with the anions. If the hydrogen ion concentration remain unchanged, the principal cations involved would be K, Mg, and Ca. By thoroughly leaching a soil, its solution may be brought to a state of very low concentration and it appears that most of the essential ions cannot attain concentrations suitable for satisfactory plant growth in the absence of the biological formation of anions.

J. C. Martin and I have performed the following experiment which bears on this point. After leaching, different portions of a soil were placed in paraffined bottles with 5 parts of distilled water, and the suspensions were shaken several hours each day for nearly a year. In several of the bottles, the water was saturated with toluene. At the end of the period of contact, only a comparatively slight amount of material had entered into solution in the case of the toluene saturated water, while in the other bottles, where microbiological processes had proceeded actively, there had been a very striking increase in the concentration of the solution in contact with the soil. Recently, these biochemical relations have been made clearer by the the studies of Burd and his associates[1] on nitrification and denitrification in relation to the soil solutes. These same investigations also give evidence of the great importance of the biological formation of SO_4 anions in soils of the type studied. Apparently, we have every reason to reëmphasize the older teachings in terms of modern soil solution theories, and it may be suggested that from this point of view the study of the microörganisms themselves must find its ultimate justification in establishing definite correlations between numbers or activities of the various types of organisms and the concentrations of the different ions of the soil solution. Likewise, it will be desirable to show that any control of the micro-population of the soil results in a corresponding control of the soil solution. It is evident that in connection with such researches, it will be of importance to conduct further investigations on the organic matter of the soil in its relation to the multiplication of different soil organisms and to the formation of nitrate, sulphate and bicarbonate anions.

BIOLOGICAL ACTION AND REPLACEABLE BASES

While the production of anions is accounted for primarily by the biological activities of microörganisms, the relative proportions of the different bases entering into solution to neutralize the acids formed are dependent to a very great extent, although not exclusively, upon the nature of those colloidal constituents of the soil involved in the replacement of bases. If these reactive compounds have had their calcium and magnesium too largely replaced by hydrogen, sodium, or trivalent bases, it cannot be expected that a satisfactory soil solution will be capable of formation, and of course, in the more extreme cases, some of the biological activities referred to above will themselves be inhibited. In the solution of the problem as a whole, it is evident that the study of biological activities and of the reactive silicates of the soil should go hand in hand. Both types of inquiry are essential in answering the basic question: Under what conditions can a soil produce an adequate soil solution? Fortunately, the development of the chemistry of replaceable bases has been very considerable and many soil problems have been clarified as a result of researches in this field. Much time also has been bestowed upon the investigation of various kinds of soil organisms, but no sufficiently comprehensive work has yet been reported dealing with these organisms and at the same time taking into account the effects of their activities on all the ions of the soil solution.

NATURE OF THE ABSORPTION OF ESSENTIAL ELEMENTS BY PLANTS

Even in these brief preliminary statements, it has been found necessary to make two assumptions, first that plants absorb inorganic elements only from the soil solution, and, second, that the absorption is primarily concerned with ions. Probably these views are held by most plant investigators, though they have been questioned by some.

Whether these assumptions are correct or not, the investigation of the soil solution is necessary, but a serious complication would be introduced if it were shown that plants possess the power of making use of soil colloids directly. Fortunately, there seems to be no reason at present to believe that this particular complication must be met.

Several misapprehensions have arisen in some discussions of soil solution theories. Some writers have confused the soil extract with the soil solution, and deduced a concentration for the latter far lower than actually occurs in a fertile soil at optimum moisture content. Moreover, plants do not absorb the soil solution as a whole, but absorb the various essential elements from the soil solution. It is a very simple matter to show that plants can absorb ions and water differentially, so that it by no means follows that the total amount of water transpired necessarily limits the absorption of ions from dilute solutions. Perhaps it may be difficult to believe that a plant can obtain enough PO_4 from soil solutions which usually contain that particular ion in very low concentration, possibly only to the extent of one or two parts per million. However, solution culture experiments have demonstrated that a concentration of PO_4 of this order of magnitude may be adequate, provided that as fast as PO_4 is absorbed, more PO_4 is added to the solution, so that the concentration never falls below a critical level during those portions of the plant's growth cycle which require active absorption of phosphate.[16] In a good soil, this is exactly the condition which prevails. With regard to the absorption of iron, experience with solution cultures has demonstrated that colloidal iron compounds will not prevent chlorosis unless conditions permit of the actual solution of a small portion of the iron.

The ionic nature of absorption, of course, is not capable of direct and certain proof by any methods so far employed, especially because the whole question of the nature of ionization is being subjected to critical review on the part of the physical chemist. But, admitted this, it still appears that the most useful conception regards absorption by plants as being concerned with ions. The differential nature of absorption, the exchange of one ion for another in a solution during absorption, the dilute character of culture solutions, the affect of one element on the absorption of another and other considerations, seem to make it profitable to interpret results in terms of ions.[13] Certainly there is no advantage (other than convenience in preparation of solutions) in referring the composition of culture solutions to the salts which were originally employed, more or less arbitrarily. By calculating in terms of milliequivalents the composition of a culture solution or the amounts of the elements absorbed therefrom, any possible inter-ionic relations will become apparent.

In pursuing this inquiry still further, it may be asked whether, in general, the concentration and composition of soil solutions from productive soils are of such a nature as to be adequate apart from the solid phase of the soil. The writer has had an opportunity to make just such comparisons by growing barley plants in artificial solutions side by side with plants grown in soils, the solutions of which were under investigation by Burd and Martin.[6] As a result of these comparisons, the question stated above can be answered in the affirmative. When a suitable amount of culture solution was used for each plant without change of solution after the earlier periods of growth, and when low concentrations of phosphate, maintained constant as far as possible, were employed, there was no difference of a really fundamental character between the artificial solutions and the soil solutions obtained by the displacement method. In both cases, the growth of the plants diminished the concentration of several of the principal ions while the concentration of HCO_3 was increased as the concentration of NO_3 decreased.

It is true, of course, that the technique of solution and sand cultures as ordinarily employed involves a somewhat different solution condition from that found in the soil, as will be pointed out later in connection with the concentration of PO_4 ions. Also, as a matter of convenience, it is frequently customary to change the culture solutions one or more times each week throughout the growth cycle of the plants. If they make good growth and if the volumes of solution are limited, it is probable that a very considerable reduction in concentration of various ions occurs during the intervals between changes of solution. This condition will be followed by a sudden change to a solution of the original concentration and the cycle will be repeated as often as the solutions are changed. In decided contrast to this condition is the one which appears to exist (at least as an average condition) in the soil solution of a cropped soil in which, notwithstanding many unpredictable fluctuations, the concentrations of several of the important ions decrease in a more or less gradual manner, and during the later stages of growth of barley and other plants, it may happen that for a considerable period of time, practically no nitrate remains in the soil solution.

ABSORPTION AT DIFFERENT GROWTH PHASES

These considerations suggest the importance of having suitable concentrations of essential ions available not merely at some time during the season, but at particular phases in the growth cycle of the plant. The work of Burd and Martin,[4] Stewart [28] and the writer,[14;17] seems to establish the fact that a very good barley crop can be obtained even when the soil solution has its concentration of NO_3 reduced to a negligible amount by the time of heading out of the plants, the fall in concentration of nitrate being accompanied by a less striking but significant fall in concentration of several other ions. As already stated, entirely analogous results have been obtained with artificial solution cultures.

It has even been suggested by Gericke,[10] on the basis of some interesting results obtained with solution cultures, that such a decrease in concentration of one or more essential elements is not only compatible with good growth, but is a necessary condition for optimum yield of crops. Although it is quite conceivable, especially in solution cultures, that an injuriously large absorption of one or more essential elements might occur or that the absorption might continue over too prolonged a period, it would be unsafe to advance at the present time too wide a generalization concerning these points, for one reason because the habits of growth of different types of plants, as well as climatic conditions, very greatly restrict the application of data obtained in any one particular experiment. One cannot disregard the question of a plant's ability to tiller or to branch in this connection. To give a specific illustration, the writer carried on an experiment with barley plants in which, because of the large volume of solution used for each plant, and the very frequent changes of solution, the concentrations of the various ions were maintained fairly constant throughout the growth cycle. The climatic conditions were very favorable, and the size of the plants grown in these solutions, in respect to total dry weight, number of tillers and amount of grain, was greater than that of plants produced during the same season by a fertile soil, even when the number of plants occupying each unit area of soil was much smaller than is customary in field practice. Likewise, the plants grown under the solution culture conditions just described were much larger than plants grown in those solutions which became reduced in concentration at the time of heading out. It is highly probable that different results might have been obtained with other types of plants possessing hereditary habits of growth which would have limited the amount of tillering or branching.

When an increased crop yield is obtained with plants like barley, by maintaining the original concentration of the culture solution throughout the season, the increase is largely dependent upon the production of successive cycles of growth, new tillers being formed over a considerable period of time and ripening being greatly delayed. (Each tiller might, of course, be regarded as a separate plant.) In general, such a situation would be entirely undesirable for plants grown under field conditions within a limited season. As a matter of fact, under a favorable climatic environment, it is very improbable that nitrate (and perhaps certain other ions) would ever be maintained in high concentration during the later stages of growth of a crop such as barley.* Burd and Martin[5] showed that even when a soil was liberally fertilized with nitrate, the concentration of this ion in the soil solution diminished during plant growth to practically as low a point as in a similar soil without treatment.

Assuming suitable moisture conditions, we may, therefore, regard it as a normal state for annual plants at least, that the concentration of the soil solution should decrease as growth proceeds, but it may be highly important, nevertheless, that appreciable, even though diminished concentrations of certain ions (for example, calcium), should be maintained in the later stages of growth, as has been shown by Gericke. It is increasingly evident that much of great value is yet to be learned about the effect of mineral elements at different stages of plant growth. The most clear-cut and convenient means of studying the question is by means of solution culture experiments. These are indeed indispensable, but the interpretation of the results of such experiments in terms of soil solution data and likewise the interpretation of data on the composition of the soil solution in terms of physiological response offer great difficulty, as I shall endeavor to point out.

PHYSIOLOGICAL NATURE OF THE SOIL SOLUTION

Earlier in the discussion, an experiment was referred to in which excellent barley plants were grown in solutions containing very low concentrations of PO_4 ion. In almost every solution culture experiment heretofore reported, solutions have been employed with an initial concentration of PO_4 far higher, in some instances several hundred times higher, than the concentrations found in the soil solutions even of productive soils which have been investigated from this point of view. Under solution culture conditions, because of this relatively high initial concentration, absorption of phosphate in the earlier stages of growth may be greater than under soil conditions and as a consequence the response to phosphate absorption in later stages of growth may be altered. At any rate, with regard to phosphate concentration, an important distinction exists between soil solutions and practically all artificial culture solutions so far described.

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* This statement may not hold for soils exceptionally high in easily decomposable organic matter.

On the basis of experiments such as those mentioned earlier, I felt justified in making the previous statement that it is possible to obtain entirely satisfactory plant growth in certain artificial culture solutions differing from soil solutions in no fundamental way. This observation, however, does not imply that the presence of a solid phase is without effect on the absorption of mineral elements from a culture solution. We have compared the composition of plants grown in sand and in solution cultures, taking care to provide in both cases exactly the same volumes of solution for each plant. The plants grown in solution culture had higher percentages of nearly all the mineral elements present than the plants grown in sand cultures, although the latter developed the larger root systems. Obviously, the ease with which diffusion takes place in a solution culture had a marked influence on the absorption of the various ions. The retarding influence on diffusion must be manifested to an even greater extent in soil media than in sand culture media. The solution in contact with the solid medium acts toward the plant as would a more dilute solution in the absence of a solid. Probably, therefore, an inhibitory concentration would be lower in a solution culture than in a sand or soil culture.

We shall now continue, in a more detailed manner, the discussion of the physiological nature of soil solutions. It is first necessary to recall that Burd and Martin[6] have been able to obtain, from certain soils, solutions which give every indication of closely approximating the soil solution as it exists in these soils at optimum or lower moisture contents. The question which interests us in the present connection is the following: To what extent does the solution displaced from a mass of soil at a given moisture content represent the solution in actual contact with the absorbing membranes of the root system of the plant. In the first place, we must recognize the possibility that not all portions of a root system are equally active at any one time. There is formed, no doubt, as growth proceeds, a constantly advancing zone of actively absorbing root cells, the older portions of the root system becoming more or less inactive at least with some plants. Composite samples of soil representing various depths of a cropped soil might, therefore, yield a composite soil solution derived from zones already more or less depleted by absorption, zones from which absorption was actively taking place and zones to which the roots had not yet penetrated. It is, perhaps, not entirely accurate to picture the soil solution of an entire mass of soil gradually becoming reduced in concentration. Possibly each absorbing root surface rapidly reduces the concentration of the soil solution immediately available, and this process continues as long as new root surfaces are formed and have access to fresh supplies of soil solution. Considering the plant as a whole, however, the supply of mineral elements would decrease as growth proceeds, if the mass of available soil were limited in amount, or if root growth ceased. In field practice, the extension of roots into deeper layers of soil and the character of the soil solution in these layers may sometimes have an important bearing on the absorption of mineral elements during the later stages of the growth cycle, as suggested by Crist and Weaver.[7]

It is difficult to say through how great distance ions can diffuse into the zone of the absorbing root membranes. In the main, the evidence indicates that it is the extension of the root system rather than the diffusion of ions to the roots which is of primary importance. Yet there must take place a considerable vertical movement of solutes along with capillary movement of water. Moreover, the application of water to the soil whether by means of rainfall or irrigation will, of course, have an important effect on the concentration of the soil solution in the various layers of the soil because of leaching processes.

While it is doubtful whether, by any method of dealing with masses of soil, it is possible to determine the exact composition of the solution in contact at any given moment with the active portions of the root system, still it can scarcely be questioned that soil solution studies are capable of demonstrating the general nature of the physiologically active solution and the tendency of plant growth to deplete such solutions. The practically complete removal of NO_3 from a limited mass of soil by barley and other crops proves that by one mechanism or another, the root system has a very efficient contact with the NO_3 ions of the entire soil solution. In this connection, it would be interesting to know how important is the differential diffusion of ions through the soil solution.

Additional emphasis should be given to the relation between the moisture content of a soil and the composition of its solution. The work so far carried out on certain California soils shows that there may exist at optimum and half optimum moisture an approximately inverse relation between moisture content and the concentration of several important ions, but this inverse relation does not hold even approximately for all ions. Especially it does not apply to PO_4 ions, which may maintain nearly the same concentration at very different moisture contents. It follows, therefore, that every change in the moisture content of the soil brings about highly significant changes in the composition and concentration of the soil solution. It is, furthermore, obvious that under the soil conditions ordinarily obtaining during the growth of a crop in the field, the same moisture content of the soil is not maintained throughout. Moisture changes might, therefore, produce changes in the concentration of the soil solution with respect to certain ions, greater than those caused by the absorption of solutes by the plant. Even under the highly controlled conditions of tank experiments, it is scarcely possible to prevent fluctuations in moisture content, especially during periods of heavy transpiration.

These considerations introduce other matters of physiological import. It appears to be quite possible to determine how a soil solution is affected by changes in the moisture content of the soil, but this does not completely answer questions relating to the physiological response of the plant to such changes. At the lower moisture content, while concentrations of solutes would be increased, rates of diffusion might be decreased. Even leaving aside the influence of the solid phase, no simple relation can be established for the physiological effects of two solutions of different concentration. Absorption studies made on such solutions have indicated that over a given period the removal of ions from the more dilute solutions may be much greater than would be predicted on the basis of relative concentrations.

In the soil, the concentration and composition of the culture solution, the moisture, and the air supply, may all influence the development of the root system and therefore the total surface involved in the processes of absorption. This naturally alters the physiological relation of the plant to the soil solution, often perhaps in a highly significant manner.

Unfortunately, the problem, so far as the absorption of ions is concerned, is even more intricate, for the reason that the absorption of any given ion cannot be evaluated except in relation to the other ions present. Thus the rate of absorption of a cation may be influenced by the rate of absorption of the associated anion and conversely. Certain ions seem to be absorbed at a slower rate than others. Among the slowly absorbed ions are as a rule calcium, magnesium, and sulphate, but such general relations are likely to vary with different types of plants. It is possible that the nitrate ion has a special position, not only because it is the source of nitrogen, but also because of its possible accelerating effects on the absorption of cations.

Clearly a knowledge of the composition of a soil solution or of an artificial culture solution does not, in itself, enable us to predict the rate at which each component ion will be absorbed or utilized by the plant.

THE SUPPLYING POWER OF THE SOIL

In any attempt to appraise the crop producing power of a soil on the basis of soil solution data, the dynamic nature of the soil and of the plant is, of course, a consideration of the utmost importance. It is essential to emphasize the supplying power of the soil, a concept which has been discussed recently by Livingston[21] in another connection The vital question is: Can the culture medium supply to the plant in each unit of time the required quota of every element needed at each particular phase of the growth cycle? According to the present view, many different soil solutions might fulfill this requirement, but obviously those of certain soils are deficient, in that the concentration of one or more essential ions falls so low that the amount absorbed in each unit of time becomes insufficient for the needs of the plant at some particular phase of growth. Theoretically, there are two general ways in which a soil might possess an adequate supplying power for essential elements: (*a*) A sufficient quantity of all the necessary elements for the total seasonal requirements of the plant might already be present in the soil solution of the total mass of soil at the beginning of the season. (*b*) The quantity of dissolved material present at any one time might be inadequate for these seasonal requirements, but additional amounts entering into solution might make up for any initial deficit. The first case can be illustrated by a sand culture in which the volume of solution and number of plants are so regulated that all elements (in suitable initial concentration) are added to the culture in the first instance in such amounts that the solution will maintain concentrations appropriate to each phase of growth. In a soil as it occurs in nature, the second method is necessarily involved, but to a degree varying for different elements. In the case of the phosphate ion, a plant presumably could obtain only a small part of its requirements for the whole growth cycle by the absorption of all the phosphate present at any given moment in the soil solution of the mass of accessible soil.

Several years ago, Burd[2] discussed this general question on the basis of data obtained on water extracts of a group of soils under intensive study. The conclusion was reached that there was always present in water-soluble form in the whole mass of available soil, a sufficient total quantity of all the various essential elements for the requirements of a large crop at any period of growth. This was true even of a soil of relatively inferior crop-producing capacity. Of course, the total quantity of water soluble material present is not the only consideration since actively absorbing root surfaces are not at all times in actual contact with the entire internal surface of the soil, but furthermore, adequate supplying power evidently means the maintenance of certain minimum concentrations of each ion in the soil solution. Just what these minimum concentrations may be in any particular instance, we cannot say. Solution culture experiments show that often extremely low concentrations may suffice, if maintained in the solution for such periods as may be required by the plant. But the conditions for diffusion are so different in a soil that we are not justified in concluding that minimum concentrations in solution cultures and in soil solutions are necessarily the same. However, in this connection, results recently published by Burd and Martin[4] are of significance. The displaced solutions of a number of soils which had become depleted through continuous cropping were examined and compared with solutions of similar soils which had remained uncropped. Even at the beginning of the season the soil solutions of the cropped soils had very low concentrations of several ions (far lower than in the soil solution of the uncropped soils). It is not certain that these concentrations would be entirely adequate in a continuously renewed artificial culture solution, and certainly in the soil, it is not at all unreasonable to suppose that they might be too low to permit of the best growth of barley under a favorable climatic environment.

Briefly recapitulating, the supplying power of a soil interpreted as a physiological function, depends upon the following factors, among others: the concentration of ions in the soil solution at the time of initial contact with the absorbing roots, the suitability of the soil for root dispersion with the consequent increase in total absorbing surface, and the ability of the soil to maintain concentrations in the soil solution above critical minima for the different phases of growth, notwithstanding withdrawal by plants. We are not in a position at present to measure directly any one of these factors. Furthermore, we do not know to what extent surplus of an element stored in a plant during an early stage of growth can supply the requirements of the plant during later stages. Solution culture studies with barley have shown that a favorable medium during early stages of growth may cause abundant tillering. If then, the supply of certain elements is exhausted too soon, none of these tillers can mature properly and the final growth is less satisfactory than if the supply of culture solution had been distributed over a longer period and growth confined to a few tillers.

The complexity of the situation might seem to render hopeless any attempt to interpret in terms of plant growth such data as can be obtained from soil solutions, yet encouraging correlations of this type have actually been obtained, even when the soil solution concentrations were computed very approximately from the results of analyses of one to five water extracts, particularly when comparisons of cropped and uncropped soils made at frequent intervals were used as a basis for estimating supplying power.

The fact of the matter is that plants possess in general a large measure of adaptability to their solution environment. It is perhaps impossible to learn the exact composition of the soil solution from which the actual absorption of ions takes place for reasons which have been set forth, but on the other hand *all the evidence at present available indicates that agricultural plants can make equally good growth in a very great variety of culture solutions.* Within wide limits at least, there is no evidence that plants thrive only in solutions with certain specific ratios existing between various elements. Davis[9] has reported definite experiments in support of this statement. While, therefore, it is true that every modification of the solution conditions is likely to induce a change in the composition of the crop, it does not follow that corresponding changes in the total crop yield will occur.

Nevertheless in cases which fall outside of this broad optimum range it is quite possible to obtain at least a general correlation between the composition and concentration of the soil solution and crop production, despite the inherent difficulties of determining the exact nature of the culture medium of a soil from a physiological point of view. It is at least reasonable to assume that considerable differences in crop production may be related to soil solution differences sufficiently striking to be made evident by the use of available methods of study, however imperfect these may be. It is only necessary that they should indicate when a decrease in supplying power for some element becomes a limiting factor, or when a toxic substance is present.

A complete discussion of the concept of the supplying power of a soil would include the oxygen and water supplying processes which are obviously of first importance and intimately bound up with the supplying power for ions. It would include also the influence of aerial conditions. No matter how self-evident, it is impossible to emphasize too strongly or too often the fact that the adequacy of a soil solution is not an independent and fixed property of that solution, but is related to the other conditions affecting the growth of the plant and the rate of the absorption of ions, such as light, temperature and humidity. Recent researches warrant the statement that insufficient attention has been given to the light factor, especially as regards duration. The limitations of this paper, however, make it impossible to more than mention these phases of the discussion.

SPECIFIC ABSORBING POWERS OF DIFFERENT TYPES OF PLANTS

One of the most interesting and important problems related to the physiological aspects of soil solution investigations concerns differences between various types of plants in regard to their ability to absorb mineral elements from the soil, sometimes referred to as the feeding powers of plants. Many hypotheses have been advanced to account for the recorded observations, but a critical review of the evidence now available convinces one that we are in possession of only a small portion of the data necessary to formulate any adequate theoretical basis for these phenomena. In any discussion of this kind, the first difficulty which presents itself is the precise meaning of the terms employed. Just what conception is conveyed by the expression absorbing or "feeding" power of a plant? It has been interpreted to mean various combinations of the following ideas:

(*a*) The difference in the percentage composition of different types of plants grown on the same soil.

(*b*) Differences in the rate of absorption of an element for each unit of surface of absorbing root membranes.

(*c*) Total quantities of an element removed from a unit area or volume of soil.

(*d*) Ability to bring into solution, by excretion of acid or disturbance of chemical equilibria, undissolved components of the soil.

(*e*) Ability to extend the root system into deeper layers of soil and thus draw on a larger total amount of soil solution.

These various interpretations may involve very different sets of processes and it will undoubtedly serve to clarify our view very much if we at least attempt to differentiate the phenomena involved.

When two types of plants are grown on the same soil, they are almost certain to have a different composition with reference to the elements obtained from the soil, but such facts do not enlighten us on the mechanism by which this difference came about. The roots of the two plants might have been in contact with surfaces of different extent, or with different soil solutions, because of modifications produced in the solutions by the vital activities of the plants. The chance that the same amount of the same soil solution would be drawn on in each case is very remote. Moreover, the composition of the plant is, of course, related to the synthesis of carbohydrates in the aerial portions of the plant, the mineral elements being diluted, so to speak, to a different extent according to the kind of metabolism possessed by the particular plant, the stage of its growth, and the local variations of the environment. Where, therefore, the composition of two plants of different types grown on the same soil is found to be different, it is an interesting fact, but, one which so far has led to no important scientific conclusions. The accuracy of the facts themselves moreover is often open to question since the soil assumed to be uniform may, in fact, possess considerable variability as well as the individual plants.

If we grow plants in artificial culture solutions in such a way that we are assured that the roots of different types of plants are in contact with the same or nearly the same solution, we may observe in many cases that different types of absorption occur, but also that the composition of a plant of any given type can be changed to a striking degree by changing the composition of the culture solution. Consequently, when comparisons are made of plants grown in the same soil, not only do we not know the exact composition of their respective culture media, but we do not know to what extent the composition of the plant reflects a specific type of absorption, and to what extent it reflects merely the kind of soil solution which happened to be available under the particular conditions and which would vary from place to place and from time to time.

Differences in the rate of absorption of an ion for a unit area of absorbing surface, cannot be ascertained, in all probability, since there is no method by which the total extent of absorbing root area can be measured. It is a matter of common observation that some plants develop much more extensive root systems than others, but these comparisons have only a very limited value for the purposes now under consideration, since the general appearance and size of a root system is not necessarily an accurate index of the total active absorbing surface.

Comparisons of the total quantities of a slightly soluble element withdrawn by different crops from limited masses of the same soil may give some idea of the relative absorbing powers of the plants for the element in question, but the values will depend upon climatic conditions, and upon the adequacy of the supplying power of the soil for other elements, and possibly upon the effects of the crops upon the development of microbiological activities.

One aspect of the absorbing power of plants which was earliest investigated had to do with the excretion of acids by plant roots. There is no doubt about the abundant excretion of carbon dioxide. The evidence of the excretion of other acids is in general negative, but this question is not yet settled. Great importance has usually been attached to the CO_2 excreted as a means of bringing into solution certain elements of the soil. Parker,[24] in a recent article, presents evidence which he believes tends to minimize the importance of CO_2 excretion. However, the experiments were conducted on one type of soil of a sandy character, and it is not certain that the use of a more highly colloidal soil would have given the same results. At any rate, it is very difficult at present not to regard the carbon dioxide excreted by plants or formed by microörganisms as of great significance, although the view of Parker may be correct that it is of less decisive influence on the comparative composition of different types of plants than has been supposed. Carbon dioxide excretion also may be concerned in another way in the absorption of ions. Nitrate ions, and possibly other anions under some circumstances, may be absorbed by many plants much more rapidly than the associated cations. The balance in the solution is maintained by the formation of HCO_3 ions. The metabolism involved in the production of carbon dioxide may, therefore, be of great consequence in this type of absorption.

The displacement of equilibria in the soil as a result of the absorption of ions by plants has been strongly emphasized in several of the best known theories regarding the relation of plants to the soil solution. It is scarcely a matter for argument that the plant does disturb the equilibria between soil mass and soil solution to a significant degree. For example, the removal of PO_4 ions by the growing plant displaces this particular equilibrium and causes more PO_4 to enter into solution. It has been suggested that the removal of calcium by a plant also plays a very significant role in the phosphate equilibrium. A certain support to this view is afforded by the experiments of Burd and Martin* in which they found that when hydrogen ion concentrations remained constant, the concentration of calcium in the soil solution had a marked influence on the phosphate concentration. As applied to the different abilities to remove phosphate from the soil, possessed by plants of different types, the critical data seem to be lacking. It should be shown, for example, that buckwheat, which is considered to have a special absorbing power for calcium, actually lowers, or tends to lower, the concentration of calcium in the soil solution to a greater degree than barley or oats.* If this is found to be true where changes in hydrogen-ion concentration do not intervene to overcome the effect, phosphate concentrations should increase and the plant having the greater ability to lower the concentration of calcium should have an opportunity to absorb phosphate from a solution with a higher concentration of this ion, or at least a larger quantity should enter into solution and be absorbed by the plant in each unit of time. In this sense and probably only in this sense, the plant would be utilizing the undissolved phosphate of the soil.

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* Burd, J. S., and Martin, J. C. Private communication.

The whole problem would be simplified if we could explain all of the relations of the plant to the soil on the basis of chemical equilibria and mass action effects. Unfortunately, biological systems do not fit completely into such a scheme. It does not seem possible to escape the conclusion that a plant cell may effect the movement of ions against a concentration gradient, involving the expenditure of energy by some mechanism as yet unexplained. Experiments on the aquatic plants Nitella and Valonia, have offered a clear picture of the general situation.

In studying the absorption of ions, it is important to recognize that a plant holds a large percentage of its inorganic elements in soluble form. Certain elements may be accumulated in a plant in large amounts without any evidence that an organic combination or a precipitation has been effected, except as regards a very small proportion of the total quantity present. On the other hand, much has been written concerning the accumulation of calcium in plant tissue in insoluble form. A striking instance of this insolubility, observed in our own investigations, is shown by the buckwheat plant, in which nearly all of the calcium may be insoluble in water. This fact, in itself, does not justify us in assuming that the insoluble calcium is necessarily in the form of calcium oxalate or similar compounds. It still remains to be determined to what extent poisonous organic acids requiring precipitation with calcium, are developed in agricultural plants. While buckwheat apparently contains nearly all of the calcium in water-insoluble form, Reed and Haas[27] have found as high as 60 per cent of soluble calcium in the leaves of citrus plants, which also are considered to have a marked power of absorbing calcium and which show great injury when the supply of calcium is too low. It has been suggested that the relatively high hydrogen ion concentration reported for the sap obtained from buckwheat plants may have an important bearing on their calcium absorbing power, but the hydrogen ion concentrations reported by Reed and Haas for citrus leaves show an intensity of acidity very similar to that of barley. These are only a few instances of the contradictions which are met with in attempting to offer any general explanation of the absorbing power of plants for ions on a basis of simple chemical equilibria.

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* Experiments of this type are now being carried out in this laboratory.

It will be well at this point to comment on the hydrogen ion concentration of expressed plant saps. It is evident that the interpretation of such data is subject to very definite limitations, because an expressed sap is a mixture derived from many types of cells, both living and dead. Changes in reaction brought about under changes in environmental conditions might result from alteration in the relative proportions of cells of different reactions rather than from changes in the reactions of cells of any given type. The surprising thing is, not that sight fluctuations of hydrogen ion concentration have been noted when plants have been grown under diverse influences, but rather that the reactions tend toward such constant values, as a general rule. A striking illustration of this is afforded by the experiments of Reed and Haas, in which citrus trees were grown in solutions of extreme types without any significant modifications of the reaction (H-ion concentration) of the sap expressed from the leaves, although the chemical composition of the tissues was influenced in a very marked way by some of the culture solutions employed.

MINIMUM REQUIREMENTS OF PLANTS

Another phase of the specific adaptations of different types of plants to the same soil solution, already mentioned incidentally, may well merit more attention. It seems to be quite true that many types of plants may grow at an optimum rate in the same kind of solution, when the composition of the solution and the rates of renewal are such that no deficiency in the supply of any element can occur. Undoubtedly, under these circumstances, some elements will be absorbed in amounts greater than necessary for growth. It is, of course, by no means true that plants absorb only what they need.

The minimum percentage of any inorganic element which can occur in a normal plant tissue will differ with different types of plants, as has been suggested, for example, by the recent work of Jones and Pember,[19] and Pember and McLean.[25] This fact might seem to afford a basis for the determination of deficiencies in culture media by the chemical analysis of plants. After accumulating a sufficient amount of data on plants grown under controlled conditions, it is reasonable perhaps to assume that some hope exists for certain correlations of this kind, yet here again we have to deal with a maze of interreacting systems. It is only necessary to mention such difficulties of interpretation as those involved in the effects of one ion on the absorption of another, the possible limited replacement of one element by another in physiological processes, and the probable effect of climatic conditions on the minimum percentages of an inorganic element capable of existing in plant tissues. Yet we have reason to believe that some plants grow better than others on a poor soil, because they can produce a greater dry weight for a given quantity of some element which exists in the soil moisture, or is renewed therein in low concentration.

Sometimes perhaps the adaptation of a plant to a poor soil is concerned with the length of the growth cycle in relation to the ability of the soil to supply essential elements to the soil moisture, and therefore to the plant. A plant with a short period of growth might be adapted to a limited supply or very early exhaustion of some essential element in the soil. On the other hand, a plant with a rapid rate of growth and a comparatively extended growth cycle might require soil in which a high rate of supply could be maintained over a longer period. Here, as in every phase of soil solution investigations, physiological problems demand study.

Probably nearly every one would agree now to the statement that a ''best'' solution does not exist for any plant in the sense in which this term is ordinarily used. In another sense a ''best'' solution or limited number of ''best'' solutions might be conceived. If each essential element could be assigned some definite value on a unit basis, somewhat after the manner of evaluating fertilizers, then the best solutions would be those producing the largest dry weights of crop for the smallest total values, corresponding to the essential elements absorbed. Practically a determination of this sort might not be feasible because of climatic and other complications referred to above. The point it is desired to emphasize is that many culture solutions of very different composition may all be equally favorable to plant growth, but some solutions may be more economical than others. If there is to be a search for best solutions, it would seem that it must be based on this idea of economy. Incidentally, it may be remarked that even if a best or most economical solution could be worked out for each phase of growth of a certain crop, it is not apparent how such a condition could be established practically in soil solutions.

The recognition of the effect of one ion on another in absorption processes does not in any way support an assumption that an element can be absorbed or utilized only in some particular ratio to another element absorbed at the same time. Nitrate, for example, may be absorbed readily from solutions of any non-toxic nitrate. It is, of course, obvious that the utilization of nitrate for growth will depend upon the adequacy of the supply of all essential elements. Thus, relatively large amounts of both potassium and nitrate may be required at certain stages of growth of cereal plants, but this is not evidence that these ions necessarily must be absorbed or utilized in chemically equivalent quantities. During plant growth, an extraordinarily complex series of chemical reactions may involve the various essential elements and we are totally unable to say at the present time how directly or how indirectly any two elements may function together in the metabolism of the plant. We can say only that lack of a sufficient quantity of some essential element may disturb the whole chain of processes or alter the internal solution environment by which metabolic reactions are influenced.

These points are discussed now, for the reason that they focus attention on the deficiency of our knowledge with reference to one indispensable phase of the study of soil and plant relations, namely, the functions of the essential elements in the synthesis of organic compounds by the plant, and the possible differences which may exist between different types of plants in this regard, either in kind or degree. Not only quantity but also quality must be considered. In general, one cannot say to what extent or why alterations in the composition of the soil solution modify the desirable qualities of the commercially important portion of a crop. The most denite information available concerns the possibility of changing the protein content of wheat by supplying nitrate at appropriate growth phases, as evidenced by the investigations of Gericke,[11] Davidson and Le Clerc,[8] and of others.

If the analysis of the problem of absorbing powers of different plants as given above is correct then we are justified in suggesting that while certain well-defined avenues of approach toward a solution are indicated, the need of the present is for data obtained under the most careful conditions of control practicable. The advancing of additional general theories may well await the results of the necessary experimentation.

Soil Acidity and Plant Growth

There is probably no phase of soil and plant relations which has received more attention than the influence of soil acidity on plant growth. Since the introduction of the hydrogen electrode into agricultural chemical laboratories, the investigation of hydrogen ion concentrations of soils has become exceedingly popular. It cannot be denied that the hydrogen ion concentration of soil solutions is an extremely important variable, one that must always be taken into account, but it should never be forgotten that many other factors may vary concurrently with variations in the hydrogen ion concentrations. If we determine this value alone, it may be a very hazardous assumption that the observed plant growth or distribution of species is correlated directly and exclusively with hydrogen ion concentration.

After all, how often do we really determine the physiologically effective pH of a soil solution? Nearly all pH values reported so far have been determined on soil suspensions. In some investigations, it has been found that within wide limits, the proportion of water to soil had but little influence on the reaction of acid soils. As the investigations are extended to include an increasing number of soils, instances are being reported in which changing the proportion of water does make an appreciable difference in the reaction of the suspension. Probably this should be expected in view of our present knowledge of soil solutions and soil extracts. The solid phase would be in equilibrium with a different solution for each proportion of water, which might result in an alteration in the amount of acid substances dissolved or in the extent of their hydrolysis. But suppose, instead of using a soil suspension, that we determine the pH of a solution displaced from a soil at a desired moisture content, are we then in a position to state that the reaction as determined is of exact physiological significance? Clearly, we are faced with the same difficulties of interpretation that have already been described with reference to the general composition of the soil solution.

The soil solution displaced from a mass of soil may have a certain definite intensity of acidity, but the question arises, are all of the absorbing root cells actually in contact with a solution of the same acidity as that possessed by the displaced solution? If the soil solution of an acid soil contains nitrate, then as plant growth proceeds, the tendency, according to solution and soil culture data, now available, would be for the solution to change its reaction in the direction of a decrease in the intensity of acidity. Therefore, if the soil solution is to exert its characteristic hydrogen ion concentration, the processes of diffusion and of solution would have to keep pace with the tendency of the plant to change the reaction of the solution. It is by no means certain that this would be the case, since a rapidly growing plant has a very marked ability to bring about changes of reaction, according to the data reported by Theron[23] and by others.

If a soil suspension shows an alkaline reaction, as determined by the usual methods, it is still more open to question whether the reaction of the soil solution immediately affecting the plant roots has the same alkaline reaction as that of the suspension, or even that of the displaced solution. In this system, the influence of the carbon dioxide given off by the plant roots, as well as that developed by the activities of microörganisms is of importance. The reaction in such systems is determined to a large degree by the equilibrium existing between CO_{3-}, HCO_{3-}, and CO_2. The percentage of CO_2 in the soil atmosphere is generally much higher than in the outside atmosphere and this higher concentration tends to reduce the alkalinity of the soil solution. In certain experiments I have found that the displaced solution of an approximately neutral soil (under crop) which had received a heavy application of calcium carbonate was slightly acid, and that the reaction became strongly alkaline after boiling the solution. The reaction of the films of solution in immediate contact with root surfaces actively producing CO_2 might have possessed a still different reaction. The fact that plants grow well in soils showing, under certain experimental conditions, a distinctly alkaline reaction in their suspensions, does not, in itself, prove that the plants make their best growth in alkaline solutions.*

If we leave aside the complications of the soil and turn to the results of solution culture experiments, we find that the preponderance of evidence does not indicate that acidity of the order of pH 5 to 6 is inimical to the growth of common agricultural crops. It is certainly true that many soils of a similar intensity of acidity are improved by liming, but various other changes occur when lime is added, besides the lowering of hydrogen ion concentration.

In the first place, the acid reaction may be indicative of an absence of calcium in the solid phase of the soil in replaceable form by which the soil solution could be replenished. Therefore, a plant might be unable to obtain from such a solution the required amount of this element. It is easy to understand that an acid soil might be unfavorable to plant growth simply on account of lack of calcium, while a culture solution of similar intensity of acidity containing an adequate concentration of calcium might present an entirely favorable medium. The importance of calcium supply in connection with acid soil solutions has been emphasized by Truog[30] and others.

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* Compare discussion by W. H. Pierre (Soil Science, 1925, xx, 285–305), published since this article was written. The data presented by Pierre emphasize a somewhat different point of view, but are not necessarily inconsistent with the opinions advanced above.

In relation to the calcium factor in acid soils, it is especially useful to interpret conditions in terms of the theory of replaceable bases, as has been done by various European investigators and by Kelley and others in America. An acid soil, during the process of its formation, may have had much of its replaceable calcium substituted by hydrogen, and thus a soil solution in equilibrium with such a system might have too low a calcium concentration from a physiological point of view. This conception emphasizes the inability of such a soil to maintain suitable concentrations of calcium in the soil solution. Of course, the nature of the plant cannot be disregarded. Some plants may grow well even in a solution markedly deficient in calcium for most agricultural plants. This adaptation may be related to some special ability for absorbing an adequate supply of calcium from a solution of exceptionally low concentration with respect to that element, or perhaps to a lower requirement for calcium on the part of the plant.

The importance of supplying the plant with an adequate concentration of calcium does not necessarily imply that the calcium must exist originally in the solution as calcium carbonate or bicarbonate for the purpose of neutralizing acids developed by the plant. Unquestionably the plant has a marked ability to develop bicarbonates from solutions containing nitrate. Since the nitrate ion may undergo complete transformation in the plant, residues of basic properties would be provided through biological processes even though the culture solution contained no carbonates or bicarbonates originally. Furthermore, in the buffer system of the plant, other cations than calcium may play an essential role. When nitrogen is supplied only in the form of ammonium salts, it may become necessary to add bicarbonate to the culture medium since the rapid absorption of ammonium tends to bring about too great a concentration of hydrogen ions.

The effect of hydrogen ion concentration on the absorption of other ions merits further investigation. Certain experiments have indicated that in complete culture solutions of an acid reaction, the total equivalents of anions (including NO_3) absorbed may exceed those of cations, the intensity of acidity in the solution being decreased usually to a point close to neutrality. With alkaline solutions, the reverse process may occur. (The excretion of CO_2 by roots, as well as the differential absorption is, of course, of primary importance in both processes.) As Reed and Haas point out, these findings may not apply to all plants nor to every ion. The investigators referred to did not find, for example, that Cl was absorbed by citrus trees more rapidly from an acid solution than from an alkaline one. It is probable that the effect of reaction on absorption is especially important in connection with absorption of nitrate ions and the carbonate-bicarbonate equilibrium. In any case, it is certainly true that no simple application of an isoelectric point theory can be made, so far as ion absorption is concerned.

Much evidence has been advanced tending to show that in addition to the effect of reaction on normal metabolism, some acid soils are inhibitive of plant growth because of toxic concentrations of aluminum or iron. Additional factors, such as the influence of reaction on microorganisms, presence of toxic organic compounds, etc., have also received attention. While attempts have been made from time to time to emphasize the complex nature of the physiological phenomena involved in the study of acid soils, universal recognition has not yet been accorded the importance of differentiating clearly between the various factors.

ALKALINE SOLUTIONS AND PLANT GROWTH

Under solution culture conditions, it has very frequently been observed that solutions of an alkaline reaction are less favorable to the growth of annual plants than slightly acid solutions. At alkalinities represented by pH 9 or above, distinct injury may occur. It is now necessary to inquire whether the unfavorable nature of an alkaline solution is solely attributable to the increased concentration of OH ions.

This is not an easy question to answer since it is difficult or impossible to maintain in alkaline solutions the desired concentration of calcium, magnesium, phosphate and iron. The fact that numerous plants make excellent growth without the slightest evidence of injury in certain acid solutions proves definitely that such concentrations of H ion are not unfavorable *per se*, but the inhibited growth in an alkaline solution does not, of necessity, prove that the hydroxyl ions are toxic. Undoubtedly the limitation of the concentrations of calcium and iron in complete culture solutions of alkaline reaction may be influential in restricting or preventing plant development. Theron,[29] however, working with a culture solution of such a composition that the reaction could be varied over a wide range, still found an alkaline reaction to be less favorable than a slightly acid reaction. Reed and Haas,[26] on the other hand, found that walnut seedlings were extraordinarily sensitive to an absence of calcium in the culture solution, and that solutions of pH 8 to 9 were not especially harmful provided calcium were present as, for example, in solutions of calcium hydrate. (However, solutions with pH values much above 9 were stated to be toxic.)

It is undoubtedly true that different species of plants have markedly different degrees of tolerance to alkaline solutions, including both the high OH ion concentration and the deficient concentrations of certain of the essential ions. In soils, a very high pH value is almost certain evidence that the soil solution has a deficiency of supplying power for one or more ions. While, therefore, much more study of the mechanism of injury is required, the emphasis on the generally unfavorable nature of highly alkaline solutions is justified.

A brief comment should be made concerning the difficulty of maintaining desired pH values in alkaline culture solutions. Once started, the plant has a striking tendency to reduce the original alkalinity, and there may be, also, an appreciable difference of reaction between the body of the solution and the solution in immediate contact with the root system. It is especially difficult to draw conclusions when sand cultures are used. In such experiments, the total volume of solution applied to the sand may be limited in amount and it is quite probable that the alkaline reaction may be reduced in intensity with extreme rapidity, especially in the films of solution surrounding the roots. It will also be found that the sand itself, however purified, tends to reduce the alkalinity of the culture solution.

METHODS OF INVESTIGATING THE PHYSIOLOGICAL EFFECTS OF SOIL REACTION

Notwithstanding the enormous volume of literature pertaining to soil acidity, experiments of the most decisive type are still lacking. Such experiments would include a very extensive series of observations on the displaced solutions or water extracts of many different soils of acid reaction. The soil solution data should be obtained, not merely for one sampling, but at intervals throughout the period of growth of several typical crops. The concentrations of the principal essential elements and of hydrogen ions should be determined and also the concentrations of aluminum, iron and manganese, as well as the oxygen supplying power (Hutchins and Livingstone)[18] of the soil, or its reduction potential as suggested by Gillespie.[12] Further application should be made of present knowledge regarding the exchange of bases. It would not be sufficient to confine the experiment to field studies, but large quantities of soil should be made homogeneous and pot or tank experiments should be arranged so as to permit of strict control of moisture conditions, aeration, etc. A properly conducted investigation, it will readily be granted, would tax the resources of an experiment station, yet the expenditure required would be but a small fraction of the sums which have been spent in the past on various field tests on acid soils. Furthermore, it is essential to have much more definite evidence than we now possess concerning the nature of the soil solutions of various acid soils which have been observed to respond in different ways to lime applications.

The numerous lime requirement methods, even though useful as empirical guides for lime application when correlated with field tests, certainly do not clarify very materially the physiological problems involved. It is no longer held that lime must always be used to the point of neutrality. Occasionally reports have been made of unfavorable effects produced by adding large quantities of lime to acid soils, and certain acid soils apparently do not require lime even for the growth of leguminous crops. Evidently a lime requirement method is of very slight assistance in analyzing the physiological condition of an acid soil solution before and after liming. What we really need to know is the quantity of lime required to bring the soil solution to a physiologically suitable composition for a given crop, not merely with regard to hydrogen ion concentration, but also with reference to the concentration of calcium, magnesium, phosphate, nitrate, or of other essential ions, also of toxic substances. All of these studies would be intimately bound up with a consideration of the effect of liming on biological activities, because of the relation of the latter to the soil solution.

ALKALI SOIL CONDITIONS

The soil conditions commonly referred to under the general term of "alkali" present, in many parts of the world, an exceedingly important special field of investigation, yet it is well to emphasize the view that the study of alkali soils is not set apart from the study of soils in general. In large measure, the general methods of attack and the basic phenomena are the same throughout. In alkali soils, as well as in other soils, we must determine the reaction, concentration, and composition of the soil solution in order to arrive at an understanding of the physiology of inhibited growth. It is true that frequently, in such soils, the physical state of the soil may be the primary limiting factor, but this condition also is to an appreciable extent determined by, or at least reflects, the character of the soil solution. In alkali soil solutions, we must sometimes take into account excessive concentrations of certain ions or undissociated salts, but it is equally important to ascertain whether any of the essential ions may not be present in too low concentration (for example, Ca or Fe), as a result perhaps of high alkalinity or of the character of the bases combined in the silicate colloids. This general question has been too fully developed in recent papers to require further discussion here. The few remarks which have just been made are intended to suggest that physiological studies of alkali soils must follow the course required of all investigations of soil and plant interrelations, including experiments with artificial culture solutions interpreted with reference to data on soil solutions.

THE NUMBER OF ELEMENTS ESSENTIAL FOR PLANT GROWTH

In the vast majority of soil and plant investigations, no attention has been given to chemical elements outside the list of those commonly thought to comprise the essential elements for plant growth. We may now regard this list as definitely proved to be incomplete. The work of Mazé,[22] the Rothamsted Experimental Station,[31] McHargue,[23] and Lipman and Sommers,* makes it necessary to include additional elements, such as manganese, boron, silicon, and perhaps numerous others, at least for the experimental conditions used by these investigators. The investigations in this field will require much extension, but the point may now be raised whether all naturally occuring soil solutions necessarily contain adequate concentrations of all of these rarer or less recognized elements, or if so, what influence on plant growth or efficiency of utilization of other elements would be produced by increasing the minute concentrations already present? It is apparent that the almost overwhelming complexities of the study of plant growth will be increased by the necessity of explaining the function of various chemical elements overlooked in the earlier history of plant investigations.

The statement may be ventured that no completely satisfactory solution of the problem of the function of any of the essential elements can be realized until the present or future discoveries of the physicist and chemist concerning the structure of the different chemical atoms are capable of being utilized by the biochemist.

POSSIBLE EXTENSIONS OF SOIL SOLUTION INVESTIGATIONS

The discussion thus far has been an attempt to present some phases of the physiological relations of plants and soils from the point of view of scientific research. Before closing, I desire to add several comments on the possibility of making practical application of researches of this type. In the first place, it will readily be admitted that there is not available at the present time any scientific method by which it can be predicted under field conditions whether or not a given soil can develop an adequate soil solution, or whether a certain system of management or fertilization will correct deficiencies. This statement, of course, does not deny the practical value of local empirical tests, especially when guided by scientific findings. Such tests are generally the best means for arriving at a decision concerning an immediate program of local soil improvement. Under the most favorable circumstances, therefore, they may be of great practical use, notwithstanding the fact that scientific reasons for observed effects may remain entirely undisclosed. The danger which inheres in field tests is that attempts may be made to generalize from them too widely. It appears that sufficient emphasis has not always been placed on the limited and local character of a majority of these tests.

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* Lipman, C. B., and Sommer, A. L. 1924. Private communication.

To what extent may it become possible to apply in the field the results and methods of intensive investigations of soils and plants, such as have been discussed in this article? Obviously, it would be useless to obtain haphazard samples of soil from the field for the purpose of studying the soil solution, because of the variability of soils and especially because of rapid seasonal changes in the soil solutions. However, it does not necessarily follow that field investigations are beyond the range of possibilities. On the contrary, it may become desirable, sooner or later, to attempt studies of soil solutions under field conditions, in selected areas in which general observation suggests that some particularly favorable or unfavorable relation exists between crop and soil solution. In each investigation of this type, it would have to be determined how samples should be taken so as to avoid any objections based on soil variability or seasonal fluctuations. These factors can never be left out of consideration, yet by the use of statistical methods and with sufficient data, it is reasonable to suppose that important correlations may be discovered. The time factor would require very careful consideration. It must be realized that a plant is not in contact with a soil solution for an hour or day only, but over the whole season and that a soil solution at one time may be completely different from the solution of the same soil at another time.

Unfortunately, the problem is made especially difficult because the character of the soil and therefore of the soil solution is not ordinarily homogeneous in different layers. In the case of certain crops, it may be very difficult to determine the exact location of the actively absorbing root system. Then, too, physical conditions in the soil may interfere with root dispersion and the limitation of growth may be chiefly a question of total available internal surface rather than of the character of the soil solution present in any given mass of soil. Certainly, it is not probable that any soil solution studies at present feasible are sufficient to explain fully the observed growth of plants on different types of soil. These remarks are particularly cogent when applied to agriculture under arid conditions where moisture relations as such are often of such critical importance.

It is not to be supposed, therefore, that intensive investigations of soils will ever entirely replace empirical tests for local guidance in soil treatment, or for the determination of the crops adapted to a given soil type. Rather, it must be the function of the controlled experiments to seek to explain the main features of crop response, to determine the cause of malnutrition, to suggest possibly new kinds of local tests and especially to establish principles which shall indicate some of the ultimate effects of the various types of soil treatment. Finally there always remains the possibility that a thorough scientific knowledge of soil and plant relations may make possible some striking practical applications at present unforeseen.

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SEX EXPRESSION IN SPINACH

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As would be expected in a dioecious, wind-pollinized plant, existing varieties of spinach are in general in a highly heterozygous condition. Commercial stocks are frequently of mixed varieties, of unsuitable types, or incorrectly named. These defects are the cause of much loss to growers and canners. Knowledge of the nature of the sexual conditions in spinach, as well as of the factors controlling sex expression, was considered essential to the work of plant breeding. There will be presented here, therefore, a description of the sexual conditions in spinach, together with results of certain experiments to test the relation of sex expression to ecological and physiological factors. The genetical aspects of sexuality in spinach will be dealt with in a later paper.

POSITION OF FLOWER CLUSTERS

After germination, spinach plants develop a rosette of eight or more leaves which arise from a much shortened stem (or "crown") located just above the surface of the ground and surmounting the thick, fleshy tap root. When the plant reaches a certain size, with the coöperation of favorable environmental conditions, the stem begins to elongate rapidly. At the same time, lateral branches arise from the axils of the rosette leaves, and in some varieties, these laterals may in time exceed the central stem in size and height. Secondary lateral branches arise from the leaf axils of both central and lateral stems. On these secondary branches, as well as on the upper portion of the central and lateral stems, are borne the flower clusters. These clusters are borne axially both on the larger stems and on the smaller branches arising from the same axils. Flowering usually begins on the middle portion of the larger stems and proceeds toward the base and the tip.

There are from six to twelve flowers in each cluster. These dev at such an unequal rate, however, that there are generally only or three in bloom at once, and the flowering period of a single clu extends over a period of from seven to ten days.

SEX ARRANGEMENTS IN SPINACH

With reference to the form of the mature plant, and to the distribution of sexes, spinach may be termed tetra-morphic. The four main classes of plants are: (1) "Extreme males." These bear only staminate flowers, and the leaves on the upper portion of the flowering branches are suppressed entirely or reduced to small scales. Representative plants of this type are shown in figures 1, 2, and 4. (2) "Vegetative males." These also bear only staminate flowers, but the leaves toward the tip of the flowering branches are more or less fully developed, as shown in figure 2. (3) Monoecious plants. These bear varying proportions of both staminate and pistillate flowers in the same cluster, and the leaves toward the tips of the flowering branches are fully developed. The ratio of staminate to pistillate flowers varies widely between the clusters on a given plant and between those on different plants. Thus a plant may be predominately

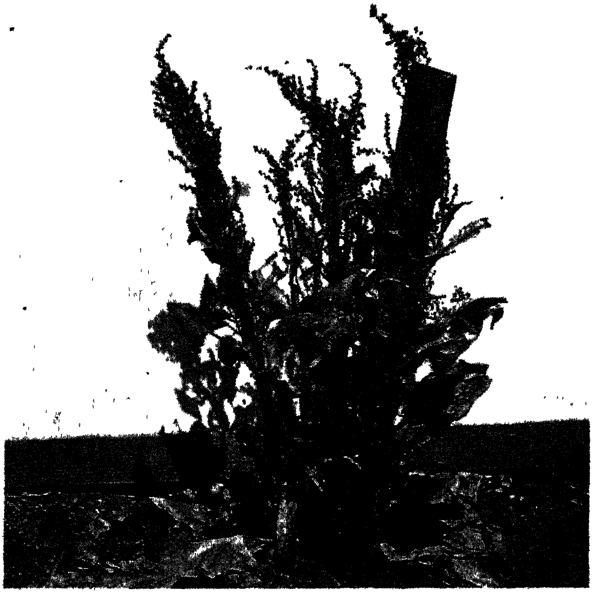

Fig. 1. "Extreme" type of male plant of Prickly Seeded spinach, in flower.

staminate, predominately pistillate, purely pistillate early in the season but with some staminate flowers later, or almost equally staminate and pistillate throughout the flowering period. The monoecious type is illustrated in figure 5. Rarely, one finds perfect flowers on monoecious plants. (4) "Female" plants. These bear only pistillate flowers and the leaves are fully developed to the tips of the stems. Females of the Prickly Seeded variety are shown in figures 3 and 4. Other varieties do not differ materially.

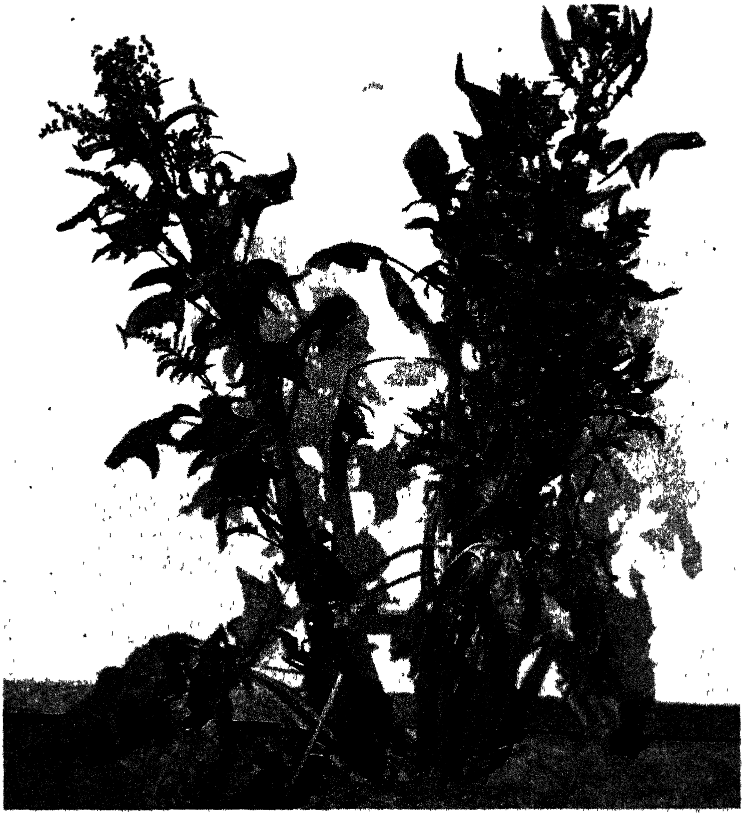

Fig. 2. Two types of male plants in the Long Standing variety. On right, extreme male. On left, vegetative male.

Of the two kinds of male plants, the extreme type is by far the more common in nearly all strains of the Prickly Seeded variety that have been grown. In other varieties, the vegetative type of male is the more common. In the Long Season and similar varieties, practically all the males are of the vegetative type. The extreme males are the first to send up their seed stalks; in fact, they sometimes do so without having formed any rosette leaves, thus producing the "spindle" type of plant figured by Kinney[8] and attributed by him to the effect of growing the plants in poor or unsuited soils. The writer's observations, however, indicate that this "spindle" condition is more probably connected with genetic factors, as is also the difference between the "extreme male" and the "vegetative male" type.

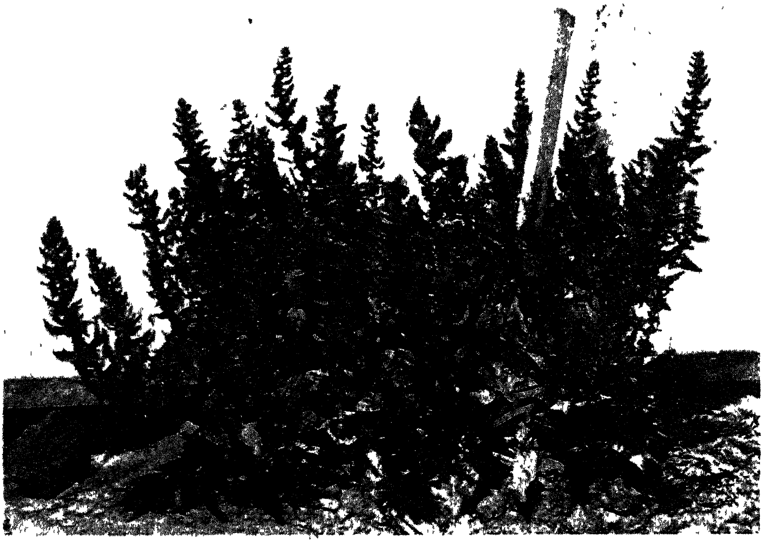

Fig. 3. Female spinach plant, in flower. When crowded the plants are taller and the development of lateral branches is less than here shown.

In 1924, a selected pistillate plant of the Prickly variety was pollinized by a vegetative male of another selection that produced males of that type only. The progeny in 1925 consisted of females and vegetative males. Strain No. 47 of the Prickly Seeded variety, which has been propagated for three years on the trial grounds of the Morse Seed Company at San Carlos, California, and has been rogued very carefully to remove all early flowering plants, now produces males of the vegetative type only, indicating that the early flowering extreme male character has been completely eliminated from the population. Commercial stocks of late or "long standing" varieties, which have been more or less carefully rogued to maintain the "long standing" character, produce few or no males of the extreme type. None of the treatments later described in this publication affected the proportion between the two types of males. All of these facts indicate that the production of two types of male plants is due to genetic factors, and not to environmental influences.

Male plants, especially the extreme type, send up their stalks earlier, begin flowering earlier, and have a shorter flowering period than females. In the progeny of a strain that had been selected for uniformity of type for two years, records were kept of the date at which each plant in a row containing 234 plants began flowering. On the male plants the average date at which the first flowers of each stalk opened was April 25, while on the females it was May 6, two weeks later. Some of the male plants began to die on May 6, and the others were nearly through flowering. By May 13, pollen had become scarce in the field. All the "extreme" males were dead on May 25, though a few of the vegetative type survived. The female plants, on the other hand, were not all in flower until May 15, and they continued growing, flowering, and forming their fruits until June 10, when they were killed by Fusarium wilt and the heat. The significance to the commercial seed grower of this disparity in flowering period will be discussed in another paper. The early appearance of the flower stalks of the male plants leads to considerable loss to growers and canners.

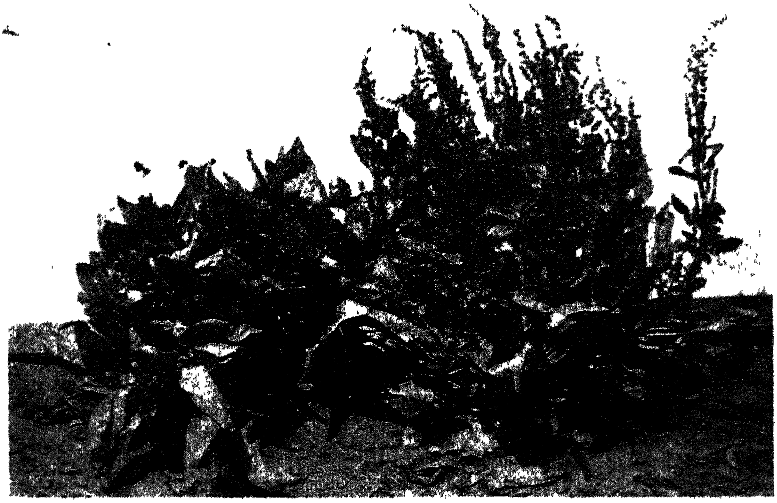

Fig. 4. Female spinach plant on left. Extreme male on right. Prickly Seeded variety.

While it is impossible definitely to identify male plants until after their stalks are well developed, it appears that in general the smaller and less vigorous individuals in the population are for the most part males. Conversely, the plants that are larger in the early part of the season may usually be later identified as females. Thus, in 1923, 13[illegible] plants of various varieties were selected, chiefly because of their large size, early in the season. Of these, 72 per cent proved to be females and 28 per cent males. As will be seen later, this is not a normal sex ratio. In the same season, a row of Prickly No. 15 was thinned somewhat when the plants were half grown. Though this strain in other tests has produced regularly a slight excess of males, in this plot of 364 plants there were 46 per cent males and 56 per cent females. Apparently, where some thinning is practiced, more than half of the remaining plants are likely to be females, because in thinning, either either consciously or unconsciously, the workman removes the smaller, less thrifty plants, which it seems are to a large extent potential males. Apparently, in spinach the extreme male plants at least, are both biologically and horticulturally inferior to the females. The general differences in size, vigor, and duration of life for extreme male and for female spinach plants agree well with those reported by Schaffner[2] and McPhee[3] in the case of hemp. The vegetative males, however, have more nearly the form and the course of development of females. The development of strains in which all the males are of the "vegetative" type seems desirable, not only because of the elimination of the non-productive, early bolting "extreme males," but because the vegetative males more nearly coincide with the females in their flowering period, thus insuring more certain pollination.

Monoecious plants are relatively rare. In many strains, none have been observed. Smith[14] reports as many as 4 per cent in some strains. The highest proportion of monoecious plants observed in a commercial stock of spinach by the writer was in Long Standing No. 16, which in one plot of 162 plants, produced 52.5 per cent males, 38.8 per cent females, and 8.7 per cent monoecious plants. Thus it seems that the monoecious plants replace females in the sex ratio; i.e., the presence of such plants nearly compensates for an abnormal deficiency of females in the population. While monoecious plants in spinach are obviously inter-sexual in their nature, and occur in varying degrees of maleness or femaleness, still the occurrence of these intergrading forms has not been affected by the widely differing environmental conditions under which spinach plants have been grown. On the other hand, seeds from monoecious plants, which may or may not have been self-fertilized, produce progenies with an abnormally large proportion of monoecious plants. Thus, eight plants grown from a monoecious individual of the Savoy variety consisted of 1 female, 3 monoecious, and 4 male plants; and 39 plants grown from seed of

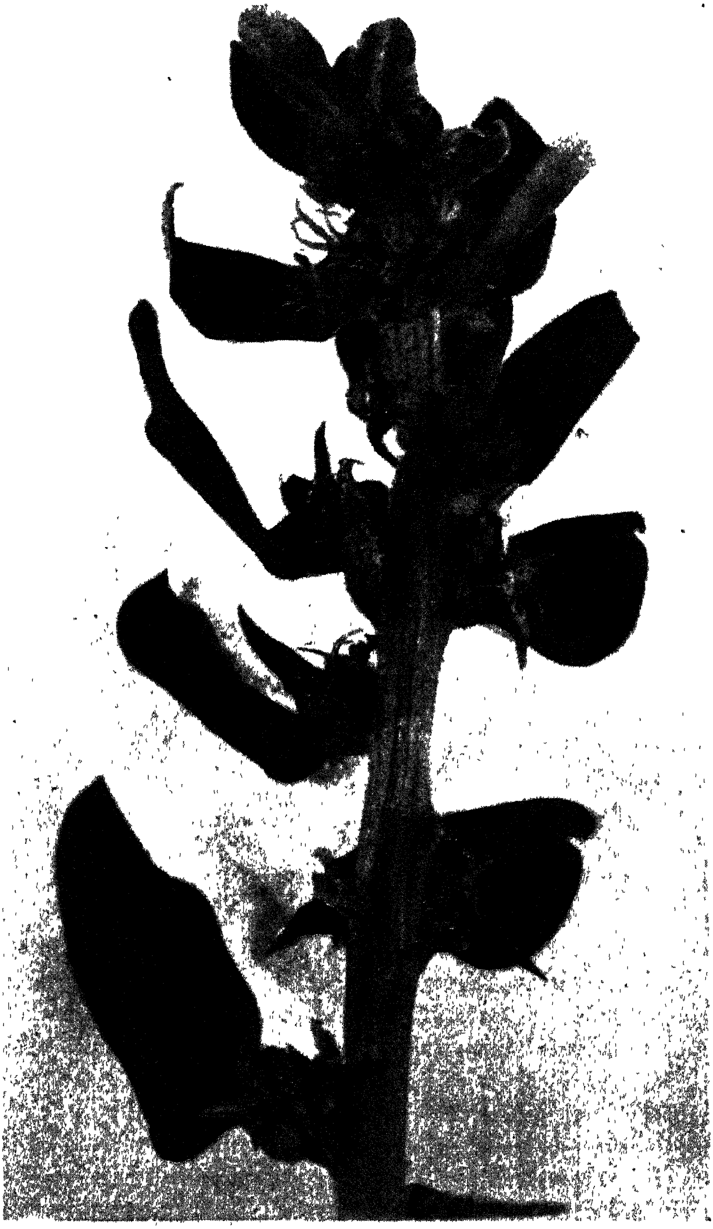

Fig. 5. Branch of monoecious spinach plant. Note the pistillate flowers and spiny seed in varying stages of development, and the anthers of staminate flowers.

a monoecious selection of the Long Standing variety consisted of 13 females, 7 monoecious, and 19 male plants. Another interesting point is that the monoecious plants in these two progenies presented the same degree of maleness or femaleness as did their respective parent plants—in one case predominately female, in the other predominately male. The evidence indicates that physiological conditions play no part in the occurence of intergrading sex forms in spinach.

There is, however, one phase of monoecism that may seem to be rather directly connected with physiological conditions within the plant. In some strains, a considerable proportion of the plants, purely pistillate in the early part of their flowering period, produce late in the season, some staminate flowers toward the tips of the branches, especially of small lateral branches. Those who believe that sexuality of plants is determined in the somatic tissues by physiological influences may consider this a form of sex reversion associated with the declining vigor of the older plants and the more adverse conditions as to temperature and moisture supply to which the plants are exposed late in the season. A similar "end-season" sex change has been observed in other plants by several writers. However, the fact that this apparent sex reversion in spinach is limited to certain strains and to certain plants within these strains, while nearby plants under the same conditions continue to be purely pistillate to the end of the season indicate that this apparent sex reversion is connected with potentialities within

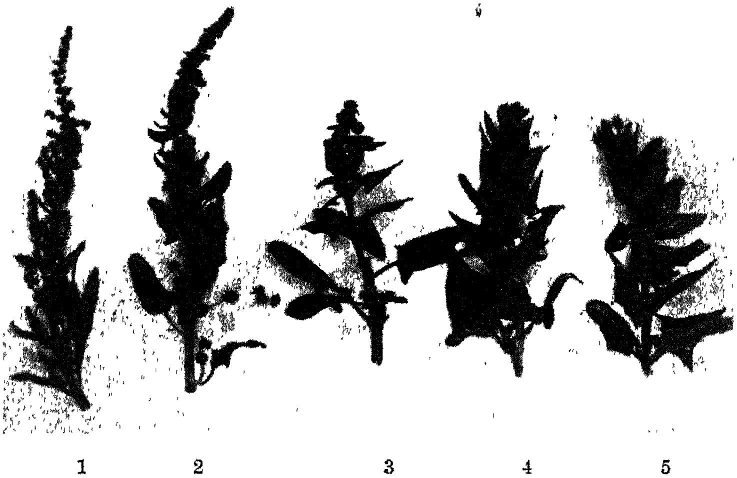

Fig. 6. Terminal branches from different types of spinach plants. (1) Extreme male, (2) an intergrading vegetative male, (3) vegetative male, (4) monoecious, (5) female.

the plant based on genetic factors. It must always be remembered that spinach, being a wind-pollinized plant, produces in even the purest commercial strains, quite heterogeneous populations, consisting of plants with widely differing genetic constitution.

THE SEX RATIO IN DIOECIOUS PLANTS

The relation of physiological factors to sex-expression has been extensively investigated in dioecious plants, though our knowledge for the basis of sex in plants is at present in a rather confused state. Much of the work reported to date indicates the possibility of physiological control of sex in plants. Thus, Halstead[6] states that in hemp grown on heavily manured soil, there was an excess of pistillate plants, while on the adjoining unmanured plot there was nearly a 1 :1 ratio. Shaded, irrigated, and check plots also produced the two forms in nearly 1 :1 ratio. Halstead also grew hemp from early, medium, and late maturing seed and observed a slight tendency for an increasing proportion of females in populations grown from the medium and late matured seed.

Correns[2] studied the effect of environmental conditions upon the ratio of hermaphroditic and pistillate flowers produced from day to day on the gyno-monoecious plants of *Satureja hortensis.* He observed an apparent connection between poor nutrition (due to poor soil, deficient light, or disadvantageous position of the plant) and a decreased proportion of hermaphroditic flowers. Under favorable growing conditions, there were only 13 per cent hermaphroditic flowers, as compared to 79 per cent under normal conditions of culture. He also observed that different strains of *Satureja hortensis* showed marked differences in the percentage of perfect and pistillate flowers. Later, Correns[3] reports that in a dioecious species of *Melanthium* there were approximately 44 per cent male and 56 per cent female plants. The sex ratio was said to be affected by varying the amount of pollen applied to the pistillate flowers. With a superabundance of pollen, 12 per cent more females were produced by the resultant seeds than from flowers receiving only a small amount of pollen. Cutting off the style soon after pollination also increased the per cent of females, indicating that female-producing pollen grains may have a more vigorous pollen tube growth or in some way effect a more rapid fertilization of the ovules. Brambell[1] concluded that every fertilized ovum must contain the potentialities of both sexes, but in dioecious organisms the fertilized egg may contain a greater inherent tendency toward one sex than the other, the chances of reversal being inversely proportional to the differences in strength of the two tendencies.

Mercurialis annua has been a favorite species for the study of sex determination and of sex inheritance in plants. However, Gillot[5] questions the work of previous investigators with this plant, on account of the irregularity of germination of the seed. Seed collected in August, 1919, gave 14 per cent germination at once, 60 per cent in 1920, 55 per cent in 1921, 60 per cent in 1922, and 52 per cent in 1923. Apparently, the preponderance of one sex over another, in this plant, varies also with the season seeds are collected, for the ratio of males to females in collections made at different seasons by Gillot varied from 84:100 to 129:100. In spinach, commercial samples of which have from 15 to 50 per cent non-viable seed, this factor might be a cause of abnormal sex ratios, if there were any difference in the viability of male or female-producing seed.

Pritchard[10] was able to alter the sex of hemp plants by several different treatments, such as bagging the tops, injecting chemicals into the stem, and particularly by removing flowers. Reversal was secured in both directions; i.e., staminate plants were caused to form some pistillate flowers, and vice versa, leading to the conclusion that both males and females are potentially monoecious. Schaffner[13] found that hemp grown in the field during summer produced pure staminate and pistillate individuals in approximately 1:1 ratio, but grown in the greenhouse in winter, it evidenced great confusion in sexual expression. Both male and female plants appeared to have potentialities of both sexes, and Schaffner was led to conclude that reversal of the sexual state takes place in the vegetative tissues. McPhee[9] also found more inter-sex types in hemp when grown in the greenhouse in winter, but concluded that while "environment in some way affects the development of sex in this species, the evidence shows that it does not control it." Although McPhee grew the plants with controlled length of day, from 3 to 24 hours illumination, his report shows no connection between this factor and sex expression of the plants.

Schaffner[14] has been able to secure complete reversion of sex in *Arisaema,* in both directions, by altering the water and plant food supply. Dryness of the soil caused all the monoecious and most of the pistillate plants to change over to staminate form. Nearly all of these plants, as well as those originally staminate, became pistillate the following year, under the influence of heavy manuring and abundant moisture. This is probably the most complete and striking case on record of sex reversion in plants.

Reide[11] has studied the correlation between the sexual condition of plants and the quotient represented by the ratio of carbon assimilation bsorption of inorganic salts. He finds that in corn this quotient st be higher for the development of pistillate than of staminate vers. Gardner[4] concluded from the evidence in the literature that aleness in plants is associated with rich soils, abundant moisture, ral spacing, the vigor of youth, favorable growth conditions in eral; maleness is associated with less favorable growth conditions. his own experiments with a normally perfect-flowered variety of wberry, Gardner observed changes in the sexual state, correlated h the widely differing nutritive conditions to which the plants were iected. Nearly all plants ''starved'' by growing in sand expressed mselves as pistillate forms, while those grown in rich soil were normally hermaphroditic. This apparent contradiction to the general rule stated above, Gardner explained by the low carbohydrate content of the starved plants at the time of fruit-bud formation, a condition that is said to be more generally associated with extreme vegetative growth under the most favorable conditions.

The only mention* in the literature of sex ratios in spinach that has come to the writer's attention is the observation of Hoffman[7] that the ratio of males to females varies according to the spacing of the plants: when crowded or more or less stunted, males predominate; with wider spacing and better growing conditions there are relatively more females. No definite ratios are given. If the wider spacing was secured by hand thinning, however, then it is possible to explain the excess of females noted by Hoffman where the plants were widely spaced, for the operation of thinning, as has been previously mentioned, is likely to eliminate the least thrifty plants, the greater portion of which are potential males.

From the facts recorded in the literature, one can hardly escape the conclusion that no general rule can explain the phenomena of sex expression for all plants. In some plants sex may be more or less directly controlled by environmental factors; in others there is some apparent environmental control of sex expression, though the principal differences are determined genetically; and in still others there is no connection between physiological factors and sex; i.e., sex is controlled entirely by genetic factors. The following experiments indicate that spinach belongs to the last class.

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* Recently, A. R. Zwaan (Seed World, 18:6, 7–9, 1925) has stated that spinach seed grown in hot and dry climates produces an excess of male plants, while seed grown in the cool moist climate of Holland produces nearly equal number of males and females. However, tests of ''place effect'' are not reliable unless the different lots came originally from the same lot of seed. The writer's tests with California, Puget Sound, and Holland seed show no consistent relation of source to sex ratio.

EXPERIMENTS ON SEX EXPRESSION IN SPINACH

It was desired to determine whether sex expression in this plant could be influenced by environment, by nutritive conditions, or by other physiological means. The value to the seed grower, of any means of regulating the sex ratio, is obvious from the fact that yield of seed per acre depends largely on the number of female plants. Observations have been made on a large number of strains, including all commercial varieties grown in this country and in Europe, as well as in the progenies of some selections. Also plots have been grown to determine the effects of a single environmental factor on sex expresion. Seeds were sown rather thickly, no thinning was practiced, and the sex of every plant was recorded.

Nutritive Conditions.—Plants have been grown in parallel plots of poor sandy soil and of heavily manured soil during three different seasons. These plots were prepared in cold frames, though the plants were grown without cover. The sandy soil was poor enough to stunt greatly the plants grown in it. Those grown in rich soil were four to ten times as large. The results of the sex counts, taken as the plants began to bloom, are given in table 1.

TABLE 1

SEX RATIOS IN SPINACH GROWN UNDER DIFFERENT NUTRITIVE CONDITIONS

Variety	Date planted	Total No.	Per cent males	Per cent females	Per cent monoecious
Long Standing No. 11	Apr. 10, 1923				
Poor soil		46	48.0	50.0	2.0
Rich soil		32	47.0	53.0	0
Prickly No. 38	Dec. 3, 1923				
Poor soil		75	46.6	53.4	0
Rich soil		121	46.3	53.7	0
Prickly No. 15	Feb. 25, 1924				
Poor soil		115	54.8	45.2	0
Rich soil		85	58.8	41.2	0
Prickly Sel. No. 10	Jan. 20, 1925				
Poor soil		190	48.4	50.6	1.0
Rich soil		202	55.0	45.0	0
Total for all tests:					
Poor soil		426	49.8	49.5	0.7
Rich soil		440	49.8	50.2	0

The combined results for all the tests being considered, it appears that there is almost exactly a 1:1 ratio between males and females. Fortunately, there were practically no monoecious plants to confuse the results. However, in the individual tests, the ratios varied slightly, fact which may be due to a tendency for certain strains to produce ore of one sex than the other, though the numbers of plants in the individual tests were hardly great enough for such small departures from the 1:1 ratio to be significant.

Shading.—Parallel plots of spinach were grown for three years, one plot being covered by unbleached "CC" muslin supported on a frame 3 feet high, the other being left uncovered. Marked differences in the size, form, and rate of growth of the plants were noted. Plants in the shaded plots were lower in per cent of dry matter, and sent up stalks and began to flower earlier. The results of the sex-counts are given in table 2.

TABLE 2

RELATION OF GROWING UNDER SHADE TO SEX EXPRESSION IN SPINACH

Variety	Date planted	Total No.	Per cent staminate	Per cent pistillate
Long Standing No. 11	May 15, 1923			
Under shade		75	48.0	52.0
Without shade		44	51.1	48.9
Prickly No. 15	Feb. 25, 1924			
Under shade		110	55.5	44.5
Without shade		90	57.8	42.2
Prickly Sel. No. 10	Jan. 20, 1925			
Under shade		197	48.2	51.8
Without shade		195	55.3	44.7
Totals:				
Under shade		382	50.5	49.5
Without shade		329	55.6	44.4

On the whole, plants grown under shade produced almost exactly a 1:1 ratio of males and females. The unshaded plants produced a slight excess of males in each case. Prickly No. 15 is a California-grown stock, extremely heterogeneous in type and the seed was of low viability. Prickly Selection No. 10 is a California-grown stock of very low viability, selected for two years for uniformity and for the long standing habit. It is the latter strain that is of special interest here for the same stock was used in other tests involving a larger number of plants and in each case produced as many or more males than females. May it be that certain strains produce seed which carry the factor for one sex to a greater extent than the other?

In any case, it seems that shading had little or no effect on sex expression, in spite of its marked effects upon the physiological activities of the plants and probably upon the chemical composition.

Spacing.—The amount of space a plant has in which to develop affects its form and activities. As compared to plants with ample room, crowded spinach plants elongate the central stem earlier, and in fact, may form no crown and rosette leaves at all; the internodes and leaf petioles are longer; axillary branches are mostly or entirely suppressed; and the flowering period begins earlier. This agrees with the commonly known fact that conditions of culture may cause quite as much difference in the form of a plant as do hereditary factors. The possible effect of plant spacing on the sex ratio was also studied. Rows were sown at the standard width of 15 inches, but with varying amounts of seed, so as to minimize the necessity for thinning to secure the desired spacing. That thinning tends to eliminate a greater number of males than of females, and so upsets the normal sex ratio, has already been mentioned. Table 3 gives the results of the more detailed experiments on spacing.

TABLE 3

RELATION OF SPACE IN THE ROW TO SEX EXPRESSION IN SPINACH

Variety	Date planted	Total No	Per cent staminate	Per cent pistillate	Per cent monoecious
Prickly No. 38:	Dec. 10, 1923				
6″ apart		330	48.2	51.8	0
2″ apart		689	50.5	49.5	0
1″ apart		645	50.4	49.4	0.2
Prickly No. 72	Feb. 19, 1925				
3″ apart		130	46.9	53.1	0
1″ apart		243	43.2	56.8	0
Prickly No. 73	Feb. 19, 1925				
3″ apart		72	48.6	48.6	2.8
1″ apart		135	45.9	54.1	0

In the spacing tests with Prickly No. 38, where large numbers were involved, almost exactly a 1:1 ratio was produced in the 1 and 2 inch spacings where practically no thinning was done. To secure the 6 inch spacing, some thinning was necessary, and here there was a slight preponderance of females. Prickly No. 72 and No. 73 proved to be stocks of identically the same strain, though secured from different sources. With this strain there was a slight though consistent excess of females, regardless of spacing, the opposite of a condition that has already been pointed out in two other strains. It appears that spacing does not affect the sex ratio in any case.

Date of Planting.—It was thought that the relation of length of day to sex expression could be studied through plantings made at intervals in winter and spring. However, it was found that the different winter plantings flowered at nearly the same time, while plantings after March 1 perished from the heat before flowering. Nevertheless, time of planting (early or late winter) does affect the vegetative development of the plants very materially. In California, November and December plantings develop very slowly during the cold weather, but grow rapidly during the warm moist periods of late winter and early spring. January and February plantings grow rapidly from the start, but do not attain as large size before stem elongation and the reproductive processes begin. The plants in a plot of Prickly Selection No. 10, sown November 15, began to flower during the period April 14 to May 14. The same strain sowed February 2, began flowering from May 1 to May 24. Nine weeks difference in planting resulted in only two weeks difference in flowering. One row of the November planting, which contained 234 plants, produced 58.6 per cent males and 41.4 per cent females. Another row planted on the same date and containing 310 plants, produced 57.1 per cent males and 42.9 per cent females. In the February planting, there were 266 plants, of which 52 per cent were males and 48 per cent females. The tendency of this strain to produce an excess of male plants has already been mentioned. The date of planting does not seem to have altered the sex ratio materially.

Mutilation.—Ten male plants that were about to flower were cut back to stubs on May 1. The small axillary branches that were left developed and produced an abundance of staminate flowers only. Ten female plants that had just begun flowering were likewise cut back. Branches that subsequently developed on these plants produced only pistillate flowers.

CONCLUSIONS

Spinach is tetra-morphic, though there are intergrading forms in the purely staminate and in the monoecious classes.

Environmental influences seem to have no effect in determining which type shall be developed. In the case of the two types of males, and in the monoecious forms, there is evidence that the differences are due to genetic factors.

Male plants, especially the "extreme males," are in general smaller, form flower stalks earlier, bloom earlier, and die earlier than female plants.

In general there is a 1:1 ratio between male and female plants, but some strains seem consistently to produce a slight excess of male plants, while others of the same variety produce an excess of females. This fact, if borne out by further tests, may be utilized through plant-breeding methods to the advantage of the seed grower, who would prefer to have an excess of females in the population.

Experiments to test the influence of rich versus poor soils, of shade versus full light, of wide versus close spacing, of early versus late planting, and of mutilation, have shown that none of these treatments have any appreciable influence on sex expression in spinach.

Thinning is the only cultural treatment affecting the sex ratio in spinach that is likely to be of any value for the seed grower. If the seeds are sown thickly and the smaller plants are rogued out early in the season, those remaining may present an excess of females.

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THE RÔLE OF ACIDITY IN VEGETABLE CANNING

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Present industrial methods of sterilizing canned vegetables of low acidity result in considerable injury to texture, flavor, and color because of the high temperatures and long periods of heating necessary to destroy heat resistant organisms. Because of the lack of proper facilities, it is not feasible in many homes, to apply the temperatures necessary to sterilize vegetables, and heating for one to three hours, at the temperature of boiling water, a method formerly recommended for home use, has been proved unsafe because it does not always destroy the spores of *B. botulinus.*

It is a well recognized fact that vegetables of high acidity, such as rhubarb and tomatoes, are easily sterilized. It is also well known that the addition of dilute organic acids to the brines used in canning makes it possible to preserve vegetables of low acidity by heating at 100° C.

However, previous investigations showed considerable variation in the effect of added acid on the sterilization of various vegetables. Not all vegetables behaved alike. Preliminary observations also showed that at least some of the variations observed could be traced to marked changes in pH value of the acidified brines during heating. Therefore, one part of the present investigation was to determine the magnitude of these changes in pH value.

A second part was to determine more accurately than had been done previously the effect of acidified brines on the sterilization of vegetables of low acidity artificially contaminated with large numbers of heat resistant microörganisms.

REVIEW OF THE LITERATURE

Experiments conducted by Cruess in 1913–1915 and reported briefly in Circular 158[1] of this station in 1916 showed that peas, string beans, pumpkins, beets, turnips, artichokes, and asparagus canned in brine acidified with 4 to 6 ounces of lemon juice per gallon and processed one hour at 212° F., kept perfectly for an incubation period of more than a year, while the same vegetables canned in the same manner but in non-acidified brines spoiled. The samples were not artificially inoculated with heat resistant organisms, but the fact that the non-acidified checks spoiled showed that such organisms were present naturally.

Dickson,[2] Burke and Ward conducted experiments in 1917 and 1918 on the effect of acidified brine on the growth of *B. botulinus* and on the destruction of its spores by heat in canned vegetables. Their conclusions are summarized in the following quotation:

> Our first series of experiments show beyond all doubt that when exposed to brines containing lemon juice in the amount of approximately 5 per cent, a virulent toxin is developed under favoring conditions of temperature. But when the spores are exposed to the action of boiling water for one hour in acid brines of similar concentrations, as in the method of canning recommended by Cruess or in a mixture of lemon juice of more than 2 per cent, as in our preliminary experiments, the spores are completely destroyed.

Their control samples, not acidified, but heated for one hour in boiling water developed growth of *B. botulinus* and a strong toxin.

Skinner and Glasgow[3] report that the addition of two tablespoons of vinegar of 4.4 per cent total acid (as acetic) per quart of brine used in canning asparagus greatly reduced the time necessary for sterilization at 100° C.

Weiss[4] determined the effect of pH value on the death point of the spores of a resistant strain of *B. botulinus*. In citric acid of a pH value of 3.16 (2.1 per cent acid) the spores were killed in less than 10 minutes at 100° C., while the time required at pH 6.66 (nearly neutral) was 90 minutes.

The same investigator[5] found that the spores of *B. botulinus* were killed at 100° C. in eleven different food juices having a pH range of 2.1 to 3.81 in less than 50 minutes, in 60 to 90 minutes in those of pH 4.22 to 4.4, in 90 to 120 minutes in those of pH 5.13 to 5.36, while those of pH 5.69 to 6.21 required 150 to 180 minutes.

Using spores of thermophiles that are extremely resistant to heat Bigelow[6] and Esty determined the death times in juices expressed from various commercially canned vegetables at 100°, 110°, 115°, and 120° C. The time necessary to kill the spores of the most resistant organism at 100° C. was as follows: 1200 minutes in corn juice of pH 6.1; 1020 minutes in pea juice of pH 5.3; 360 minutes in sweet potato juice of pH 5.0; 210 minutes in spinach juice of pH 5.0; 360 minutes in string bean juice of pH 5.0; 210 minutes in beet juice of pH 4.7, and 210 minutes in pumpkin juice of pH 4.5.

Dickson[7] and his associates in 1922 reported experiments in which the heat resistances of *B. botulinus* spores in various concentrations of hydrochloric, citric, acetic, and lactic acids were determined. At corresponding pH values, there seemed to be little difference in the toxic effect of the various acids. The OH ion also exerted a toxic effect, as it was found that the heat resistance was much less in alkaline than in neutral solutions.

Esty[8] and Meyer conducted an extensive series of experiments to determine the effect of hydrogen ion concentration on the death point of *B. botulinus* in phosphate solutions, Difco pepton solutions, double strength veal infusion, spinach juice, and in juices expressed from many canned foods. Because of the action of buffer substances, much more acid was required to give the desired pH values in spinach juice than in phosphate and peptone solutions.

The spores in a citric acid solution of pH 5.26 were killed in 65 minutes at 100° C., at pH 4.69 in 40 minutes; at pH 4.31 in 20 to 25 minutes, while at pH 7 about 330 minutes was required.

Bigelow[9] and Cathcart in studying the changes in hydrogen ion concentration occurring during the processing of many canned foods found that the pH value decreased slightly in most cases on account, they believed, of the coagulation of buffer substances and the formation of weak acids through decomposition of organic compounds by heat. In one experiment, however, that with beans in tomato sauce, there was an increase in pH value in the sauce which the authors state was "due to diffusion of acid into the beans." Undoubtedly, part of the change was due to this cause; but we believe, from our experiments with other products, that some of it may have been due to the buffer action of compounds leached from the beans during processing.

CHANGES IN HYDROGEN ION CONCENTRATION DURING CANNING

The foregoing review of the literature shows that a number of investigators in several laboratories have studied the effect of pH value on the death point of heat resistant organisms, but that little has been published on the changes occurring in the pH value of acidified brines in canned vegetables during actual canning and sterilizing operations. Unless the extent of these changes is known, it is impossible to specify what the hydrogen ion concentration of the acidified brine should be at the time of its addition to the product. Therefore, in our investigations, we gave as much attention to this phase of the problem as to tests on the effect of pH value on the death point of heat resistant spores.

1. *Procedure.*—Various vegetables were prepared for canning in the usual manner and as described later. All of the brines used in the experiments, except those for corn, consisted of 2 per cent of salt in distilled water plus various concentrations of hydrochloric, citric, or acetic acids. The brine for corn contained 2 per cent of salt and 5 per cent of sugar in addition to the added acid.

The pH values of the solutions when prepared for use in the canning tests were carefully checked colorimetrically by the Clark and Lubs[10] method against standardized buffer solutions of known pH values.

In the first year's experiments number 2 cans were used, filled to the usual height with the same weight of vegetables. They were then filled with the brine, but this was not measured. In later experiments 8-ounce cans were used. Weighed amounts of vegetables and measured amounts of brine were added. Eighty grams of sweet corn plus 125 c. c. of brine; 120 grams of string beans plus 100 c. c. of brine; 180 grams of spinach plus 25 c. c. of brine; and 130 grams of asparagus plus 90 c. c. of brine were the ratios used. The later experiments, therefore, can be more easily duplicated.

The filled cans were heated in live steam at 99° to 110° C. for 5 to 8 minutes before sealing, the time varying with the size of container and character of the product.

"Sanitary" (open top) cans were used and were sealed with a hand roll, foot pressure, power driven double-seamer. Tests showed that the sealing operations were satisfactory.

"Processing" in all investigations reported in this publication was conducted with boiling water at approximately 100° C. Various time periods varying from one-half to two hours were used.

After exhausting and again after processing for the various times, samples of the brines from the cans were taken, filtered, and their pH values determined colorimetrically.

2. *Relative Changes in pH Value during Exhausting and Processing.*—Changes in pH value of acidified brines in cans of vegetables were greater during exhausting before sealing than during processing in the sealed cans. All acidified brines increased in pH value during exhausting and most brines increased during processing.

Table 1 illustrates the relative changes that were observed in several experiments during exhausting and processing. The table presents only a small proportion of the data obtained.

TABLE 1

TYPICAL PH VALUE CHANGES DURING EXHAUSTING AND PROCESSING OF BRINES ACIDIFIED WITH CITRIC ACID

Vegetable and pH value of its juice	pH value of original brine	pH value of brine after exhausting 5–8 minutes	pH value of brine after 60 minutes processing at 100° C.
Sweet corn, pH 6.8	2 8	3.6	4.2
Sweet corn, pH 6.8	4 0	5.6	5 2
String beans, pH 6.2	2.8	3.6	3.8
String beans, pH 6 2	4.0	5 8	5.4
String beans, pH 6.2	6.0	6.0	5.4
String beans, pH 6.2	7.0	6.0	5 3
Spinach, pH 6.8	2.8	3.0	4.0
Asparagus, pH 5.4	2.8	3.9	4.0
Asparagus, pH 5.4	3.6	4.6	4.5

Possibly in those acidified brines in which the pH value increased during exhausting but later decreased during processing, buffer substances were precipitated or decomposed, or acids or acid salts were formed. Continued increase of pH value during processing was probably due to diffusion because it is probable that during exhausting most of the buffer effect occurred from compounds dissolved from the vegetables.

3. *Effect of Length of Processing on pH Value.*—Several vegetables were processed for 20, 40, 60, and 80 minutes in order to determine the effect of the length of processing at 100° C. on pH changes in acidified brines. Some of the data are presented in table 2.

After exhausting and the first 20 minutes of processing, changes in pH value were slight in most instances.

TABLE 2

EFFECT OF LENGTH OF PROCESSING ON CHANGES IN pH VALUE OF BRINES ACIDIFIED WITD CITRIC ACID

Vegetable and pH value of its juice	pH value of original brine	pH value after processing at 100° C.			
		20 minutes	40 minutes	60 minutes	80 minutes
Sweet corn, pH 6.8	2.0	2.9	3.0	3.2	3.2
Sweet corn, pH 6.8	2.8	4.0	4.2	4.2	4.2
Sweet corn, pH 6.8	4.0	5.4	5.2	5.2	5.2
Sweet corn, pH 6.8 (Not acidified)	7.0	6.5	6.2	6.2	6.1
String beans, pH 6.2	2.0	3.2	3.2	3.2	3.0
String beans, pH 6.2	4.0	5.4	5.4	5.4	5.4
Asparagus, pH 5.4 (1924)	2.0	3.6	3.5	3.2	3.1
Asparagus, pH 5.4 "	3.6	4.4	4.4	4.5	4.6

TABLE 3

CHANGES IN pH VALUE OF ACIDIFIED BRINES DURING PROCESSING AS AFFECTED BY TYPE OF ACID

Vegetable	Acid used	Original pH value of brine	pH value of brine after 1 hour at 100° C.
Asparagus (1923)	None	7.0	5.6
Asparagus "	Hydrochloric	2.6	5.4
Asparagus "	Citric	2.6	5.1
Asparagus "	Acetic	2.6	4.6
Asparagus "	Hydrochloric	3.6	5.6
Asparagus "	Citric	3.6	5.6
Asparagus "	Acetic	3.6	5.4
Sweet corn	None	7.0	6.2
Sweet corn	Hydrochloric	2.0	5.0
Sweet corn	Citric	2.0	3.2
Sweet corn	Hydrochloric	4.0	5.6
Sweet corn	Citric	4.0	5.2
String beans	None	7.0	5.4
String beans	Hydrochloric	2.0	4.8
String beans	Citric	2.0	3.2
String beans	Hydrochloric	2.8	4.4
String beans	Citric	2.8	3.8
String beans	Hydrochloric	4.0	5.4
String beans	Citric	4.0	5.4
Peas, green	Hydrochloric	2.6	5.8
Peas, green	Citric	2.6	5.4
Peas, green	Acetic	2.6	4.8
Peas, green	Hydrochloric	3.0	6.0
Peas, green	Citric	3.0	5.7
Peas, green	Acetic	3.0	5.4

4. *Comparison of Different Acids.*—A considerable number of experiments were made to determine the relative changes in pH value occurring in brines acidified with hydrochloric, citric, and acetic acids, respectively. In table 3 some of the data obtained are presented.

In brines of relatively low original pH value, acetic acid gave pH values after processing that were lower than those acidified with citric acid, and citric acid in turn lower than hydrochloric acid. These observed differences are very probably due to the differences in degrees of ionization of the different acids.

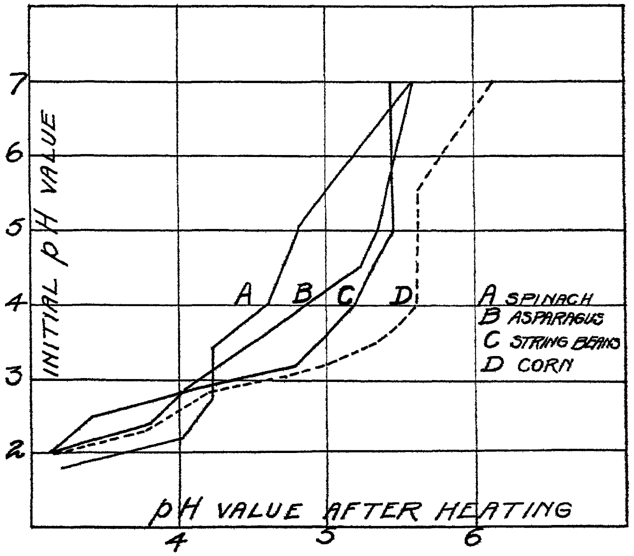

Fig. 1. Changes in pH value of acidified brines during processing at 100° C.

For practical purposes, citric is to be preferred to acetic acid because it does not affect the flavor so noticeably.

Three per cent of lemon juice added to brine gave a pH value of 2.9 before processing and a pH on carrots after processing of 5.0. For a 5 per cent addition of lemon juice, the figures were 2.7 and 4.8, respectively. A brine acidified to pH 3.0 with citric acid increased to 5.2 during processing on carrots. Apparently the change during processing is about equal with brines of equal original pH value whether citric acid or lemon juice is used.

5. *Comparison of Different Vegetables.*—Typical changes in pH value in brines on asparagus, corn, string beans, and spinach are shown in tables 3 and 4 and figure 1. There was some variation in pH

changes in different lots of the same vegetable, probably occasioned by differences in their chemical composition; e.g., see asparagus in tables 4 and 5. Usually this variation did not exceed .2 pH.

TABLE 4

RELATIVE EFFECT OF DIFFERENT VEGETABLES ON CHANGES IN pH VALUE OF BRINES ACIDIFIED WITH CITRIC ACID. (See also table 3.)

Vegetable	pH value of original brine	pH value of brine after processing 1 hour at 100° C.	Ratio of vegetable to brine
Carrots	3.0	5.2	2 : 1 approx.
Carrots	3.8	5.4	2 : 1 approx.
Carrots	4.4	5.4	2 : 1 approx.
Carrots	4.8	5.4	2 : 1 approx.
Carrots (check)	7.0	5.4	2 : 1 approx.
Peas (green)	3 0	5.7	2 : 1 approx.
Peas (green)	3.6	6.0	2 : 1 approx.
Peas (check)	7.0	6.0	2 : 1 approx.
Artichokes	2.6	4.6	1 : 1 approx.
Artichokes	3.0	4.8	1 : 1 approx.
Artichokes	3.6	5.3	1 : 1 approx.
Artichokes (check)	7.0	5.3	1 : 1 approx.
Sweet corn	2.0	3.2	0.64 : 1
Sweet corn	2.8	4.2	0.64 : 1
Sweet corn	4.0	5.6	0.64 : 1
Sweet corn	7.0	6.2	0.64 : 1
String beans	2.0	3.2	1.2 : 1
String beans	4.0	5.2	1.2 : 1
String beans	6.0	5.4	1.2 : 1
String beans (check)	7.0	5.4	1.2 : 1
Spinach	2.8	4.0	7 : 1
Spinach	3.4	4.2	7 : 1
Spinach	3.8	4.4	7 : 1
Spinach	7.0	5.6	7 : 1
Asparagus	2.0	3.2	1.4 : 1
Asparagus	2.8	4.0	1.4 : 1
Asparagus	3.6	4.5	1.4 : 1
Asparagus	5.0	5.4	1.4 : 1
Asparagus	7.0	5.4	1.4 : 1

Evidently peas possess a higher concentration of buffer substances than do the other vegetables. It would appear that the other vegetables named in the table exert about an equal effect on the pH value of acidified brines, at least on brines of low to moderate pH value. At high pH values differences are more pronounced.

EFFECT OF PH VALUE ON THE DEATH POINT OF RESISTANT BACTERIAL SPORES

Experiments were conducted with three heat resistant organisms, *B. sporogenes, B. botulinus,* and a thermophile and with six varieties of vegetables, asparagus, artichokes, sweet corn, peas, spinach, and string beans in order to obtain information on the effect of pH value on the death points of these organisms under practical canning conditions.

1. *Procedure.*—In the preliminary experiments in 1917 and 1918 brain medium cultures of four strains of *B. botulinus* from Dr. I. C. Hall of the Bacteriology Department, University of California, were mixed and used for inoculation of canned peas, corn, and string beans. Each culture contained abundant spores, but their heat resistance by test tube tests was not determined.

In the 1923–24 experiments, 4-day old cultures of a heat resistant strain of *B. sporogenes* grown at 37° C. were used; and 3-day old cultures in the 1924–25 experiments. The cultures were diluted 1:3 with sterile water and 1 c. c. of the diluted culture used to inoculate each 8-ounce can of vegetable.

A resistant strain of a thermophile was obtained from Dr. J. R. Esty of the National Canners' Research Laboratory, Washington, D. C., through the courtesy of Dr. K. F. Meyer. It was grown at 55° C., on nutrient agar slants and the organisms were washed from the agar with sterile water to give a rich suspension of spores. One c. c. of the suspension was used for each 8-ounce can of vegetable.

A limited number of tests were made also with a suspension of resistant *B. botulinus* spores furnished by Dr. K. F. Meyer. This suspension in our experiments was diluted 1:5, and 1 c. c. of the diluted suspension was used for inoculation of each 8-ounce can.

In a special meat medium of approximately pH 7, the *B. sporogenes* spores used in the 1923–24 experiments survived 180 minutes at 100° C., but were killed in 195 minutes. In 2 per cent glucose broth of pH 7 they survived 165 minutes at 100° C., but were killed in 180 minutes.

The *B. sporogenes* spores used in the 1924–25 experiments were killed in 165 minutes in the meat medium and in 135 minutes in the glucose broth at 100° C.

The thermophile spores were killed in from 315 to 350 minutes in glucose broth of pH 7—the resistance varying somewhat with the cultures used. The *B. botulinus* spores survived 275 minutes but were killed in 300 minutes at 100° C., in glucose broth of pH 7.

As a further check on the heat resistance in non-acidified media, inoculated but non-acidified samples of the canned vegetables were prepared in each experiment. In order to enable us to interpret our results on heat resistance more intelligently the rates of heat penetration in 8-ounce cans of experimentally packed string beans, corn, asparagus, spinach, and commercially canned creamed corn at 100° C., were measured by means of thermocouples.* Figure 2 gives the results of these measurements.

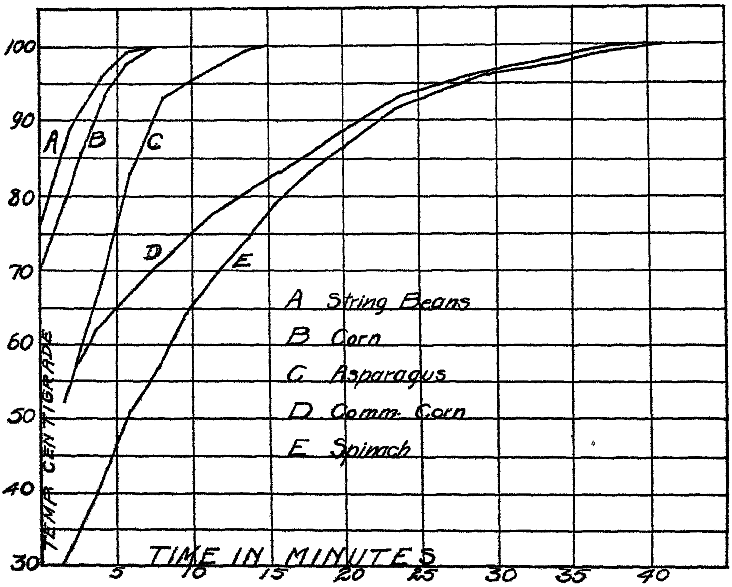

Fig. 2. Heat penetration in 8-ounce cans of string beans, corn, asparagus, commercially canned corn, and spinach.

Heat penetration in spinach was extremely slow, about 40 minutes at 100° C. being necessary for the center of the can to reach 99° C. The other vegetables tested reached the temperature of the bath very quickly.

2. *Effect of Acidified Brines on Heat Resistance of B. sporogenes.*—The data can be most conveniently considered according to the variety of vegetable used.

(*a*) *Asparagus:* Tests with this vegetable were made during both the 1923–24 and the 1924–25 seasons. The data are given in table 5. Three cans were used for each concentration in the 1923–24 experiments and four cans for each in the 1924–25 experiments.

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* These tests were made in coöperation with J. Parcell.

The asparagus was cut to fit the cans, washed and then blanched 5 minutes in boiling water. It was not graded for size. The spores were added before the can was sealed and before exhautsing. The cans were then filled with brines of the desired pH values, exhausted 5 minutes in live steam and sealed immediately. They were put at once into boiling water and heated for the length of time shown in table 5. Number 2 cans were used in the 1923-24 season and 8-ounce cans in the 1924–25 season.

The cans were chilled completely to room temperature in cold water after removal from the processing vessel and then incubated at 37° C. for 3 months in the 1923–24 experiments and about 2½ months in the 1924–25 experiments. Spoiling was evidenced by swelling of the cans, but was confirmed by examination of the contents of the cans and by making transfers to nutrient media.

The data of the two seasons agree in indicating that for brines of equivalent pH value, acetic acid is somewhat more effective than citric in lowering the heat resistance of *B. sporogenes.* The ratio of volume of brine to weight of asparagus was greater in the 1924–25 tests, which explains the smaller changes in pH values and lower indicated heat resistance in the 1924–25 tests. The 1923–24 ratio, however, more nearly represents commercial practice.

In a can tightly packed with asparagus, apparently an original pH of 2.6 or less is necessary to insure destruction of the *B. sporogenes* spores used in 2 hours at 100° C.

When the pH values after processing are compared there is found a closer correlation between the results of the two seasons' experiments. Apparently the critical pH value if determined after processing is about pH 4.6, as the organism failed to grow after 1 hour's processing at 100° C. in both seasons' tests, when the final pH value was 4.6 or less.

(*b*) *Artichokes:* Artichokes inoculated with *B. sporogenes,* canned in plain brine of pH 7 and processed 1 hour at 100° C. spoiled. Those canned in citric acid brines of pH 2.6, 3.0, and 3.6 and processed 1 hour at 100° C. did not spoil. The final pH values were 4.6, 4.8, and 5.3, respectively.

(*c*) *String Beans:* Growth of *B. sporogenes* failed to occur in string beans after 1 hour's heating at 100° C., in the 1923–24 experiments, even in non-acidified brine, although the pH value of the non-acidified brine after processing was 5.4. In the 1924–25 experiments, therefore, the processing periods were shortened to ½ and 1 hour respectively.

TABLE 5

EFFECT OF pH VALUE OF BRINE ON CANNED ASPARAGUS ON HEAT RESISTANCE OF *B. sporogenes*

Acid	pH value of original brine	pH value of brine after processing	Period of processing at 100° C. in hours	Per cent of cans spoiled
1923-24 SEASON				
Hydrochloric	2.6	5.4	1	0
Hydrochloric	2.6	5.4	2	33
Citric	2.6	5.1	1	33
Citric	2.6	5.1	2	0
Acetic	2.6	4.6	1	0
Acetic	2.6	4.6	2	0
Hydrochloric	3.0	5.5	1	100
Hydrochloric	3.0	5.5	2	33
Citric	3.0	5.4	1	66
Citric	3.0	5.4	2	0
Acetic	3.0	5.2	1	33
Acetic	3.0	5.2	2	0
Hydrochloric	3.6	5.6	1	100
Hydrochloric	3.6	5.6	2	100
Citric	3.6	5.6	1	100
Citric	3.6	5.6	2	66
Acetic	3.6	5.4	1	0
Acetic	3.6	5.4	2	0
None (check)	7.0	5.6	2	100
1924-25 SEASON				
Citric	2.0	3.1	½	0
Citric	2.0	3.1	1	0
Citric	2.4	3.4	½	0
Citric	2.4	3.4	1	0
Citric	2.8	4.0	½	0
Citric	2.8	4.0	1	0
Citric	3.6	4.6	½	25
Citric	3.6	4.6	1	0
Acetic	3.6	4.6	½	0
Acetic	3.6	4.6	1	0
Citric	4.5	5.2	½	50
Citric	4.5	5.2	1	25
Acetic	4.5	5.2	½	25
Acetic	4.5	5.2	1	25
Citric	5.0	5.3	½	50
Citric	5.0	5.3	1	50
Acetic	5.0	5.3	½	25
Acetic	5.0	5.3	1	0
None	7.0	5.4	½	100
None	7.0	5.4	1	75

Citric acid only was used for acidifying the brines. Spoiling did not occur even after boiling for only ½ an hour when the original pH was 3.2 or less and the final pH 4.8 or less. When brines of original pH 3.6, 4.0, and 4.5 were used, spoiling occurred after boiling for ½ an hour but not after 1 hour. At pH 5.0 or above spoiling occurred in all cases.

Evidently some factor other than pH value affects the heat resistance of *B. sporogenes* in string beans, because the spores were more quickly killed in string beans than in asparagus in brines which were of the same pH value after processing. Nevertheless the critical pH value—between 4.8 and 5.0—was not greatly different from that found for asparagus.

(*d*) *Sweet Corn:* Fresh sweet corn on the cob was purchased in the local wholesale market, husked, cut from the cob, canned in 8-ounce cans, inoculated as previously described and brines of various pH values were added. The brines in one series were acidified with hydrochloric and in another with citric acid. The amount of brine used was greater than in commercial practice in order to obtain rapid heat penetration (see figure 2).

Spoiling did not occur in cans to which citric acid brines of pH 3.2 or lower were added, whether boiled 1 or 2 hours. When brines of pH 3.6 or higher were used spoiling occurred in all cases after boiling for either 1 or 2 hours except at pH 3.6, heated for 2 hours. The pH values after processing were 5.0 and 5.4, respectively.

With hydrochloric acid brines, the results were similar. The critical pH value of the brine after processing appeared to be about pH 5.0 to 5.2, somewhat higher than with asparagus and string beans.

(*e*) *Peas* (1923–24 season): The peas were prepared for canning as in regular cannery practice by shelling and blanching in water. They were placed in number 2 cans and each can was inoculated with 10 c. c. of a suspension of a 4-day old spore culture of *B. sporogenes*—a heavier inoculation than was used for corn, string beans or spinach. Brines of three pH values and acidified with acetic, citric, and hydrochloric acids, respectively, were used (see table 6).

The critical pH value after processing was, for peas with a 1½ hour processing period at 100° C., about 5.4 with both citric and acetic acids. This corresponded to an original pH value of 3.0 for acetic acid and 2.6 for citric acid. The increase in pH value was greater with peas than with the other vegetables.

As in other experiments the critical pH value is affected by the length of processing.

(*f*) *Spinach:* Spinach from the local wholesale market was trimmed, washed, blanched five minutes and canned in 8-ounce cans, 180 grams per can. It was inoculated with 1 c. c. of a suspension of a 3-day old *B. sporogenes* spore culture and 25 c. c. of brine was added per can. In one series citric and in another acetic acid was used (see table 7).

TABLE 6

EFFECT OF ACIDIFIED BRINES ON HEAT RESISTANCE OF *B. sporogenes* IN CANNED PEAS. (1923–24 SEASON)

Acid	pH value of original brine	pH value of brine after processing	Period of processing in hours	Per cent of cans spoiled
Hydrochloric	2.6	5.8	1	100
Hydrochloric	2.6	5.8	1½	100
Citric	2 6	5.4	1	0
Citric	2.6	5.4	1½	0
Acetic	2.6	4.8	1	0
Acetic	2 6	4.8	1½	0
Hydrochloric	3 0	6.0	1	100
Hydrochloric	3.0	6.0	1½	100
Citric	3.0	5.7	1	100
Citric	3 0	5.7	1½	100
Acetic	3 0	5.4	1	33
Acetic	3.0	5.4	1½	0
Hydrochloric	3.6	6.0	1	100
Hydrochloric	3.6	6.0	1½	100
Citric	3.6	6.0	1	100
Citric	3.6	6.0	1½	100
Acetic	3.6	5.6	1	100
Acetic	3.6	5 6	1½	66
None (check)	7.0	6 0	1½	100

The results with acetic acid were virtually the same as with citric acid.

Heat penetration is slow in spinach, a condition that may account for some of the spoiling of samples of relatively low pH value. Thus spoiling occurred at pH 4.2 (referring to brine after processing). However, even allowing for heat penetration, decreased pH value of the brine was less effective in spinach than in the other vegetables studied. Probably the leaves matted together in the can and prevented penetration of the acid to all the spores.

Because of this latter possibility another experiment was made. Spinach was blanched in brine acidified with citric acid in one case and with acetic in another and was canned in brines of pH 2.0

to 6.0. The canned samples were inoculated heavily and processed 1 and 2 hours at 100° C. Spoiling did not occur in any sample. This experiment suggests a method of applying acidified brines in spinach canning commercially.

3. *Effect of Acidified Brines on Heat Resistance of Spores of a Thermophile.*—The spores of this organism withstood 315 minutes at 100° C. in glucose bouillon in a control test, but were killed in 350 minutes.

TABLE 7

EFFECT OF CITRIC ACID BRINES ON THE HEAT RESISTANCE OF *B. sporogenes* SPORES IN CANNED SPINACH

pH value of original brine	pH value of brine after processing	Period of processing at 100° C. in hours	Per cent of cans spoiled
1.8	3.2	1	0
1.8	3.2	2	0
2.2	4.0	1	0
2.2	4 0	2	0
2.8	4.2	1	25
2.8	4.2	2	0
3.4	4.2	1	100
3 4	4.2	2	75
4.0	4.6	1	100
4.0	4.6	2	100
5.0	4 8	1	100
5.0	4.8	2	100
7.0	5 6	1	100
7 0	5.6	2	100

Fresh corn was prepared and canned as describeed for the *B. sporogenes* experiments. Brines acidified with hydrochloric and with citric acids were added. The cans were processed at 100° C. for 1, 3, and 5 hours. Since the pH changes during processing have been given elsewhere the detailed data from this experiment will not be presented.

Where the pH value of the brine acidified with citric acid was greater than or equal to 5.4 after processing, spoiling occurred even after 5 hours boiling. At pH 5.2 and less, spoiling did not occur. With hydrochloric acid, spoiling occurred at pH 5.5 but not at 5.4, indicating that hydrochloric acid may be more toxic than citric.

One striking observation was that processing for 1 hour was as effective as 5 hours, when the critical pH value was reached.

4. *Effect of Acidified Brines on Heat Resistance of Spores of B. botulinus.*—The experiments with *B. botulinus* have not been so extensive as with *B. sporogenes*. However, enough was done to con-

firm in general the results reported by Meyer, Esty, Weiss, Dickson, and others (see review of literature).

Preliminary experiments were made in 1917 and 1918 in order to compare the "cold-pack-one-period" and the "lemon juice" methods of home canning. Four strains of *B. botulinus* growing in brain medium were mixed, shaken violently with sterile water, and centrifuged. Shaking with sterile water and centrifuging were repeated twice in order to remove some of the toxin and to break up the clumps of medium. Spores were numerous in all four cultures.

For some of the tests commercially canned vegetables were used; for others, the fresh vegetables. The canned and glass packed samples were inoculated heavily with the mixed spore suspension; heated at 100° C. for one hour, except check samples, and then incubated for 2½ months. Check samples were inoculated but not heated. Their appearance and odor were then determined and 1 c. c. of the liquor, obtained by crushing the vegetables with the brine and straining the resulting "purée," was used for subcutaneous injection of guinea pigs.*

No growth or toxin formation occurred in string beans either acidified or non-acidified and whether heated or not.

Heavy growth, gas production and toxin formation occurred in peas when unheated; when not acidified and heated 1 hour at 100° C. and when canned in brine containing 4 per cent lemon juice but not heated. No growth or toxin production occurred when they were canned in brine acidified with 4 per cent lemon juice and heated for 1 hour at 100° C.

Corn heavily inoculated with the spores and heated 1½ hours at 100° C., with brine containing 6 per cent lemon juice did not spoil and was not toxic.

Since peas were found to be an excellent medium for growth of *B. botulinus*, another experiment was made for direct comparison of the "one-period-cold-pack" and the "lemon juice" methods of home canning. Canned peas were re-canned, heavily inoculated and treated as follows:

a, no acid and not heated; *b*, brine containing 6 per cent lemon juice but not heated; *c*, same as *a* but placed in water in wash boiler and brought to boiling for 3 hours; *d*, same as *b* but heated at boiling point 1 hour; *e*, same as *d* but quart mason jars used instead of cans. The cans and jars were incubated for 1 year at 37° C.

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* The guinea pig inoculations were made by Dr. J. Traum of the University of California, Veterinary Science Division.

All cans in lots *a, b,* and *c* spoiled and developed the characteristic *B. botulinus* odor. Lots *d* and *e* did not spoil. Ten cans or jars were used for each test.

This test indicates that the "lemon juice" method is much safer than the "cold-pack-one-period" method of home canning, even when only 1 hour at 100° C. is used in the former and 3 hours at 100° C. in the latter.

We realize that the spores used were not so resistant to heat as some spore cultures later developed by Meyer, Dickson, Esty, Burke, and others. Nevertheless a survival of 3 hours at 100° C. is an evidence of marked resistance to heat.

A spore suspension of *B. botulinus* from Dr. K. F. Meyer was used recently for a series of tests with asparagus and spinach. These spores, according to Dr. Meyer, were able to survive 300 minutes at 100° C., in a medium of pH 7—when at their maximum resistance. Their maximum resistance at pH 7 in glucose broth as determined in our laboratory at the time of our experiments was 275 minutes. However, the incubation period of the heated tubes was not long enough to permit the delayed germination of a few spores that may have survived 275 minutes.

Brines of pH 2.0, 2.4, 2.8, 3.6, 4.5, 5.0, 6.0, and 7.0 were used for the asparagus and brines of pH 2.2, 2.8, 3.4, 4.0, 5.0, and 7.0 for the spinach. One set of brines acidified with citric acid and one with acetic acid was used with each vegetable.

At each pH value four cans were heated at 100° C., for 1 hour, four for 2 hours, and four for 3 hours.

The asparagus developed typical *B. botulinus* spoiling after 1 hour of processing but not after 2 or 3 hours when the pH value after processing had dropped to 4. The same results were obtained with spinach. However, the period of incubation has been less than three months—further incubation may change the results.

In order to produce a pH of 4 or less after processing it was necessary to add to the asparagus at the time of canning a brine acidified with citric or acetic acids to pH 2.8 or less and to the spinach a brine of pH 2.2 or less or to blanch the spinach in acidified water before canning.

The percentage of cans spoiling was irregular. Similar results were observed in the other three experiments with asparagus and spinach.

While the experiments with *B. botulinus* were not so extensive as those with *B. sporogenes,* nevertheless they show that *B. botulinus* is much less resistant to heat in cans of vegetables containing acidified brines with a final pH value after processing of 4.0 or less than in non-acidified brines under the same conditions. Apparently the critical pH value for *B. botulinus* is lower than for *B. sporogenes* or the thermophile used in these investigations.

SUMMARY AND CONCLUSIONS

1. Brines of relatively low original pH value increased in pH value during exhausting by heat and during processing by heat for moderate lengths of time. This increase was much greater than could be accounted for by diffusion and we are forced to conclude, therefore, that it was caused principally by the action of buffer substances dissolved from the vegetables.

2. The change was greater during exhausting than during subsequent processing.

3. Brines of high initial pH value decreased in pH value, possibly because of formation of weak organic acids such as CO_2, H_2S, etc., and by precipitation of buffer substances.

Brines of low pH value increased in pH value to a maximum during the first part of the heating process; then on further heating decreased in pH value, perhaps for the reasons just given.

4. The increase in pH value of acidified brines in canned vegetables was less in brines acidified with citric and acetic acids than in those acidified with hydrochloric acid, because of difference in buffer effects with these acids.

5. The change in pH during heating was greatest with peas and least with artichokes.

6. The effect of the pH value of the brine on the heat resistance of the spores of *B. sporogenes* and *B. botulinus* and a heat resistant thermophile was very pronounced.

7. The pH value during the heating period, practically equivalent to that found after heating, was found to be more significant in relation to the effect on the heat resistance of spores than was the initial pH value of the brine because the initial pH value changes greatly during heating.

8. Spinach canned with a small amount of acidified brine (ratio of vegetable to brine 7:1) exhibited irregularities with respect to effect of pH value on heat resistance of *B. sporogenes;* probably because the brine failed to reach all parts of the rather tightly packed mass of leaves. However, preliminary blanching of the spinach in acid brine of pH 3 made it possible to easily sterilize it at 100° C., even when heavily inoculated with *B. sporogenes* spores.

9. The results of these investigations cannot be applied directly to commercial canning operations until large scale experiments under factory conditions are made. Nevertheless they show that brines acidified with a small amount of citric or acetic acid greatly reduce the heat resistance of the spores of heat resistant bacteria and that if the decrease in pH value during heating is taken into account, it is possible to sterilize canned vegetables much more easily in acidified brines than in non-acidified brines.

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FACTORS GOVERNING THE INITIATION OF SPROUT GROWTH IN CITRUS SHOOTS*

BY

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I. INTRODUCTION

It is a well known fact that in many plants the removal of a portion of a vertical shoot results in the outgrowth of buds which otherwise would have remained dormant. This outgrowth also occurs when a vertical stem is changed to a horizontal position. This phenomenon is generally termed regeneration or reconstitution. The term regeneration is applied in this paper to the outgrowth of buds when this results either from the removal of a part or from the change of position of a stem.

In most cases this outgrowth on vertical shoots is confined to the buds in the uppermost region, the length of the sprouts declining steadily as the distance from the apex increases. In horizontal shoots the outgrowth is confined to the dorsal side, the buds on the ventral side remaining dormant. This dominance or subordination is commonly referred to as physiological correlation or simply correlation.

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With many plants, when an isolated piece of stem is placed under favorable conditions, sprouts will grow from the apical and roots from the basal end. This type of response is supposed to be due to polarity of the stem.

The investigations here reported deal with regeneration of vertical and horizontal Eureka lemon (*Citrus Limonia* Osbeck) shoots and Chinese lemon (*Citrus Medica* var.) cuttings. Both species are evergreens and their growth habits are similar. The tendency of young trees, or of old trees which have been pruned severely, is to produce long, succulent, vertical shoots. So long as the tip grows vigorously these shoots remain unbranched but when, toward the end of the growing season, the rate of growth decreases, laterals appear near the apex. If a shoot is bent permanently into a horizontal position, laterals are produced along the dorsal side. The shoots of both species exhibit very strikingly the phenomenon of polarity in regeneration, hence this material was used for the purpose of studying the factors involved.

II. THEORIES OF REGENERATION

Before proceeding with an account of these investigations it may be profitable to outline the outstanding hypotheses which have been advanced to explain the phenomena mentioned above. Vöchting[23] concluded from his extensive studies that polarity in vegetative shoots is primarily due to unknown internal influences. External factors like gravity, light and moisture may modify polarity to a limited extent but the internal influences persist from generation to generation.

Sachs[22] explained polarity by postulating the existence in stems of shoot-forming substances that migrate upwards and root-forming substances that migrate downwards.

Loeb[13] explained the results of his earlier experiments with *Bryophyllum calycinum* in the sense of Sachs' theory, namely that the "flow of (specific?) substances in the plant determines when and where dormant buds or anlagen shall begin to grow."

From later studies,[14] however, Loeb concluded that there is an inhibitory substance produced at the stem apex which flows in the direction of the basal buds, and he believed that the reason the uppermost bud grows out first when the stem is cut from the mother plant is that this bud is first freed from inhibitory substances.

Subsequently Loeb practically renounced the hypothesis of an inhibitory substance and concluded[16] on the basis of other experiments with Bryophyllum that "a simple mass relation can be used as a guide through the bewildering maze of the phenomenon of regeneration." This mass relation is that equal masses of isolated tissue of the same type exposed to the same external factors produce about equal masses of regenerated roots and shoots in equal length of time. The inhibiting action of one part of a tissue upon another he explained by stating that the sap starts to flow to the group of bud anlagen which begin to grow first and that the other part of the tissue remains dormant because of the continuous flow of sap to the growing part. Loeb mentioned two possible explanations of polarity, first, that there might be a chemical difference between ascending and descending sap which determines the nature of growth and, second, that the anlagen reached by the ascending and descending sap are primarily different. He considers the second alternative the most plausible since he was able to get regeneration of roots with both ascending and descending saps.

Goebel[7] explained that the reason why regeneration takes place when a stem is cut is that the nutritive materials accumulate below the cut.

Curtis[5] suggested that "inhibition of shoot growth at nodes below the terminal one may be due to a lack of sufficient food and to inability to compete successfully for water rather than to a backward flow of some inhibitor." In a previous paper[4] he reported results with sucrose solutions which he cites as an example of reversal of polarity. On the basis of unpublished results he claims to have found other substances which are even more efficient than sucrose in the reversal of polarity.

Robertson[21] believes that the growth-influencing substance concerned in regeneration is the nuclear autocatalyst, which is formed in the cell nucleus and escapes into the pericellular fluid during mitosis. In any given cell, division is regulated by the relative volumes of nucleus and cytoplasm or by what he terms the nuclear-cytoplasmic ratio. Every cell then contains endogenous autocatalyst which is retained within the nucleus and exogenous autocatalyst which has escaped into the pericellular fluid. He shows mathematically that if the exogenous supply is large, then the endogenous supply is small and vice versa. Hence if the supply of exogenous autocatalyst is large, only a small supply of endogenous autocatalyst can be produced before equilibrium is imposed upon the reaction of nuclear synthesis, and if this does not lead to cell division then no further multiplication of this type of tissue is possible. But if the supply of exogenous autocatalyst is small, then the endogenous autocatalyst can escape into the pericellular fluid and renewed synthesis of nuclear material becomes possible.

It will be seen that Robertson supports the general theory held by Sachs, Goebel, Loeb, etc., that polarity is brought about by the transportation of actual substances. His own theory, however, is more specific than those advanced by others like Sachs and Loeb, for he actually ascribes the rôle of inhibitor and accelerator to one and the same substance, the nuclear autocatalyst.

Child[3] believes that the theory of formative stuffs or transportative correlation has little real explanatory value. He does not deny the existence of transportative correlation but he maintains that it cannot exist until the different systems are present; for example, the flow in opposite direction of shoot and root-forming substances as postulated by Sachs cannot occur autonomously but becomes possible only when regional differences of some sort are present which determine the flow. Child assumes that dominance is effected by the transmission of energy-changes or excitation, rather than by the transportation of chemical substances. This assumption is based on his theory which will now be considered.

In all organisms there are gradations in the intensity of metabolic processes which determine the fundamental outlines of axial symmetry and structural differentiation. Centers of high metabolism like the head of a planarian worm or the apex of a stem tend to dominate or control centers of low metabolism. Dominance then depends primarily upon the rate of metabolism and seems to operate by impulses, excitations, or changes transmitted in various ways from the dominant region to other parts. Although the primary difference between dominant and other levels of the gradient is purely quantitative, yet quantitative changes may, sooner or later, bring about differences in constitution and character of the protoplasmic substratum. Each level of the gradient develops a characteristic protoplasm and the character of the protoplasm in turn alters or modifies the characters of the reaction. In this way, different specific substances may be produced at different levels of the gradient and chemical transportative correlation then becomes possible. Gradients may be reversed or obliterated or new gradients established by environmental agencies which change the metabolic rate in different parts of the organism, but the gradient once established, persists through asexual and perhaps also through sexual reproduction.

In an earlier publication[2] Child discussed the relation of dominance and subordination between different parts of the root system. The results of certain experiments with roots obtained by Goebel and McCallum indicate to him that "not only does a relation of dominance and subordination exist between the different parts of a root system, but that the root system as a whole dominates the stem to a certain extent, so far as the production of roots is concerned. If this dominance and the dominance of the stem-tip both result from metabolic gradients, then there must be in plants possessing roots two metabolic gradients in opposite directions, the apical region of one being in the stem-tip or tips, that of the other in the root-tip or tips." This is impossible unless the two gradients have different paths of transmission or are of different metabolic character. Child therefore conceived the possibility that the inhibiting influence of the roots upon the stem may be a transportative rather than a transmissive correlation and that it becomes ineffective when this transportation decreases to a certain minimum or when the two parts are separated. But the apparent dominance of the root system over the aerial part of the plant with respect to root formation is a secondary relation and hence, according to Child, is dependent upon the primary relation which is transmissive in character.

III. APICAL DOMINANCE IN VERTICAL SHOOTS AND CUTTINGS

A vigorous vertical lemon shoot generally remains unbranched during the entire growing season. If laterals are produced they are mostly confined to the apical region. When such a shoot is cut back to the mature wood, laterals will appear only from buds close to or immediately below the apex of the remaining portion. The number of sprouts produced depends upon the vigor of the mother shoot but ordinarily not more than seven are formed. At first these sprouts grow at about the same rate, but within a few weeks the growth rate of those nearest to the apex is accelerated while that of the subapical sprouts slows down; generally growth ceases after the end of the first cycle. Every healthy bud below this active region can be forced to produce a vegetative sprout by notching or girdling above the bud or by mechanically preventing the apical portion of the shoot from producing sprouts.

The investigations here reported were undertaken for the purpose of studying apical dominance in the Citrus stem. For the work with cuttings the Chinese lemon was chosen because it can be grown more successfully by this method than other species of Citrus. The investigations in the orchard were carried out with shoots of the Eureka lemon. All material was obtained from trees growing on the grounds of the Citrus Experiment Station, Riverside, California.

(*a*) CHINESE LEMON CUTTINGS

In the course of preliminary experiments it was found that if the upper half of a cutting (about 30 cm. in length) was enclosed in a plaster cast and suspended in moist air, sprouts would appear immediately below the cast. When the plaster cast was removed and the cutting again suspended the uppermost buds grew out, while within a few weeks the original sprouts died. The results of this experiment were discussed by Reed and Halma[20] and were considered to support the inhibitor theory advanced by Loeb.[14] The results of the following experiments, however, make it evident that the initiation of sprout growth cannot be explained on the basis of the inhibitor theory.

Chinese lemon cuttings, each possessing ten buds, were divided into three sets. In one set of 31 cuttings the cut surface and the three uppermost buds were wrapped tightly with rubber tape (bricklayer's tape); in another set of 31 cuttings the cut surface and the five uppermost buds were wrapped. A set of 15 unwrapped cuttings served as a control. The pressure of the wrapping with tape mechanically prevented development of the buds. The cuttings were then planted vertically to a depth of two to three centimeters in flats containing washed river sand and placed in the greenhouse. In order to increase the humidity of the surrounding air the flats were covered with glass cases.

Measurements of the sprouts produced by each cutting were made every three or four days. The tape was removed when the total length of the sprouts produced by the free portion had reached various lengths. After growth ceased the green weight of sprouts and roots was determined. The data obtained are given in tables 1 and 2 and they are graphically represented in figure 1.

TABLE 1

CHINESE LEMON CUTTINGS; SHOWING THE EFFECT OF TAPING THE THREE UPPERMOST BUDS

Cutting No.	Total length of subapical sprouts		Total length of apical sprouts, cm.
	When apex was freed, cm.	At end of experiment, cm	
5	0	22	8
24	1	26	7
19	2	22	12
31	3	29	8
15	3	24	12
21	4	21	8
12	4	25	11
25	6	17	22
26	6	17	3
30	7	16	12
16	7	20	14
6	8	21	13
23	8	19	30
17	9	20	5
11	9	23	21
28	10	16	9
22	10	23	7
20	10	17	10
7	11	20	9
27	11	22	9
13	13	28	7
18	14	22	9
29	14	19	6
8	16	18	5
10	16	18	6
1	16	19	24
14	16	23	11
2	17	20	1
4	17	18	16
9	18	31	8
3	21	21	6

TABLE 2

CHINESE LEMON CUTTINGS; SHOWING THE EFFECT OF TAPING THE FIVE UPPERMOST BUDS

Cutting No.	Total length of subapical sprouts		Total length of apical sprouts, cm.
	When apex was freed, cm.	At end of experiment, cm.	
18	1	19	15
21	1	12	10
22	2	17	10
31	2	19	11
29	2	18	13
27	2	13	7
24	2	18	11
10	2	19	19
16	3	18	12
6	4	21	18
26	4	14	9
23	4	23	20
20	4	27	14
12	5	18	11
15	5	13	8
25	5	16	36
28	5	14	15
30	5	14	29
17	6	19	10
5	6	24	14
4	7	18	7
19	8	20	12
11	10	22	7
8	10	18	12
7	11	27	4
13	11	13	9
14	13	15	10
9	18	23	12
1	19	22	8
2	23	25	24
3	26	34	29

In contrast to the results obtained in the experiment mentioned previously,[20] none of the subapical sprouts died. It is important to mention that the control cuttings began to grow from two to three days earlier than the other two sets.

It should be noted that even where no measurable growth was made by the free portions of the cuttings before the tape was removed the ultimate growth compared favorably with that made by other subapical regions which had a better start.

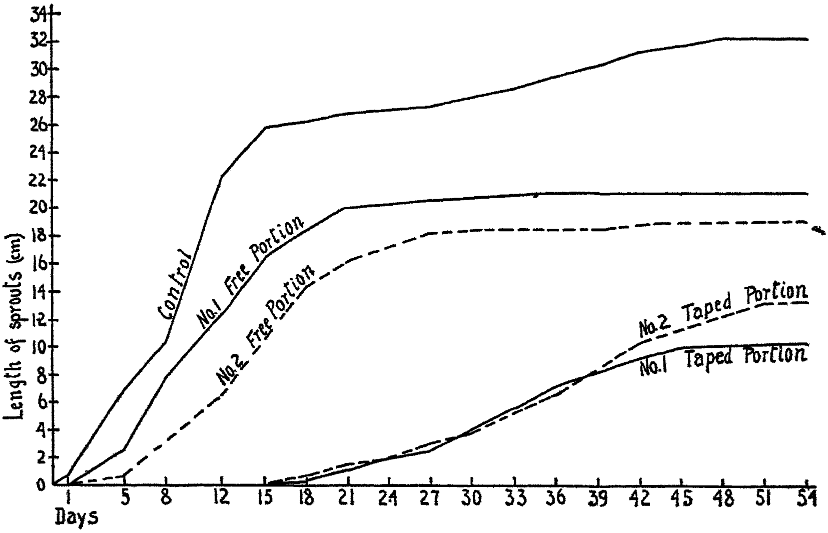

Fig. 1. Chinese lemon cuttings. Average total sprout growth per cutting. No. 1, three uppermost buds taped; No. 2, five uppermost buds taped. Control not taped.

Figure 1 shows that the growth of both free and inhibited portions of the cuttings follows the characteristic S-shaped curve. This suggests the possibility that the two portions, although connected morphologically, behave as two physiological units.

Before examining this possibility it is necessary to discuss the relation between size of cutting and amount of sprouts produced. This is of importance to the interpretation of the data obtained with taped cuttings. During the winter 58 cuttings of various sizes were made from the mature part of 14 mother shoots of the Chinese lemon. They were grown about the same time and under the same conditions as the cuttings in the experiment described above. For the purpose of finding out whether or not a difference existed between apical and basal portions of the mother shoot, the cuttings from each were kept in the order of their position on the mother shoot.

TABLE 3

CHINESE LEMON CUTTINGS; RELATION BETWEEN SIZE OF CUTTING AND AMOUNT OF SPROUTS PRODUCED FROM INDIVIDUAL CUTTINGS OF FOURTEEN MOTHER SHOOTS

Weight = total green weight in grams of sprouts per 100 gr. of green weight of cutting.

Length = total length in cm. of sprouts per 100 sq. cm. of area of cutting.

In the "Individual" column, the apical cutting is given first.

Weight		Length		Weight		Length	
Individual	Average	Individual	Average	Individual	Average	Individual	Average
20.0		81.2		28.0		70.3	
30.4		64.7		34.2		57.8	
31.5		54.2		25.5		68.1	
37.5	29.9	61.6	65.3	25.8		68.1	
				38.9	30.5	59.5	64.8
35.2		51.1					
27.6		73.8		36.4		70.3	
32.6	31.8	60.4	61.7	33.3		52.6	
				31.3		56.8	
45.3		71.4		33.3		55.1	
43.9		90.9		35.3	33.9	58.4	58.6
43.1	43.1	80.0	80.7				
				54.1		62.5	
35.6		70.0		36.7		69.9	
35.7		81.1		49.3		73.4	
47.4	39.9	73.1	74.7	41.1		73.8	
				40.1	44.3	61.2	68.2
29.4		52.6					
24.5		68.1		46.8		74.6	
20.7		62.9		39.1		73.3	
30.6		43.3		38.3		71.4	
27.1	26.5	38.0	53.0	44.0	42.0	66.8	71.5
44.2		71.1		43.1		83.3	
47.3		69.0		48.3		66.6	
47.9	46.4	76.0	72.0	47.4		62.0	
				46.8		68.5	
38.9		55.1		38.5	44.8	57.1	67.5
26.3		72.7					
22.9		35.1		43.1		55.1	
36.9	31.2	73.9	59.2	34.5		48.9	
				34.7		60.0	
36.1		62.4		29.4	35.4	61.2	56.3
27.8		80.0					
28.4		68.8					
25.0		69.0					
22.5	28.0	62.5	68.5				

The green weight and length of the sprouts were determined after six weeks when growth had stopped. In table 3 the total length of sprouts is given on the basis of 100 sq. cm. of stem area of the cuttings on which they grew and the green weight of sprouts on the basis of 100 grams of green weight of stem of the cuttings on which they grew. The average green weight of sprouts produced by each set of cuttings varied from 26.7 to 46.4 grams—a difference of *19.7 grams.* The maximum variation in sprout production among the cuttings from the same mother shoot is *17.5 grams* and in the entire set of 58 cuttings is *34.1 grams.*

The correlation coefficient between green weight of cuttings and green weight of sprouts produced is .806 ± .032 and that between stem area and length of sprouts is .747 ± .040. The inclusion of the roots does not alter these values materially.

With these relations in mind we shall now examine the sprout production by the free and the temporarily inhibited portions of the cuttings. Table 4 gives the average amount of growth produced by the free and the inhibited part of the cutting respectively. It will be seen that in the case of cuttings whose uppermost three buds were taped the free parts produced 25.0 grams of sprouts and the inhibited part 42.7 grams—a difference of *18.7 grams.* It was stated above that the greatest difference between cuttings of the same mother shoot was *17.5 grams* and for the entire lot *34.1 grams* (table 3). In view of the fact that the cuttings wrapped with tape were parts of many mother shoots it is evident that, had the two portions of the cuttings been separated, a similar variation in the production of sprouts would have occurred. It is therefore probable that the difference between free and taped portions was due to variation of the material.

TABLE 4

CHINESE LEMON CUTTINGS; MASS RELATION OF SPROUTS ON TAPED AND FREE PORTIONS AS COMPARED WITH SIMILAR RELATIONS ON CONTROLS

Number of buds temporarily taped	Free portion		Taped portion		Entire cutting	
	Green weight of sprouts (grams) per 100 grams of green weight of stem	Length of sprouts (cm.) per 100 sq. cm. of stem area	Green weight of sprouts (grams) per 100 grams of green weight of stem	Length of sprouts (cm.) per 100 sq. cm. of stem area	Green weight of sprouts (grams) per 100 grams of green weight of stem	Length of sprouts (cm.) per 100 sq. cm. of stem area
3 (31 cuttings)	25.0±1.21	65.6±1.85	42.7±3.39	63.7±3.89	28.7±1.23	65.4±1.56
5 (31 cuttings)	36.3±1.35	62.4±1.75	32.6±2.56	65.1±3.17	33.4±1.51	62.9±1.94
Control (15 cuttings)	......	......	......	......	27.7±1.52	59.1±1.20
Control (58 cuttings)	......	......	......	......	35.8±0.75	65.1±0.95

The cuttings whose five uppermost buds were temporarily inhibited produced 36.3 grams from the free portion and 32.6 grams from the inhibited portion—a difference of only *3.6 grams.* As regards length of sprouts produced the values for both sets of cuttings are so close that no explanation is needed.

The figures in table 4 indicate that temporarily inhibiting a part of the apex resulted in the division of the cutting into two physiological units each of which produced sprouts in proportion to its mass of the cutting. If this is correct then the ratio of sprouts to unit weight or area of stem should be approximately the same for each set regardless of the number or location of the sprouts. This is actually the case. For example, the cuttings whose three uppermost buds were taped produced 28.7 grams of sprouts per 100 grams of cutting, the other set 33.4 grams, and the two sets of controls 27.8 and 35.8 grams respectively (table 4).

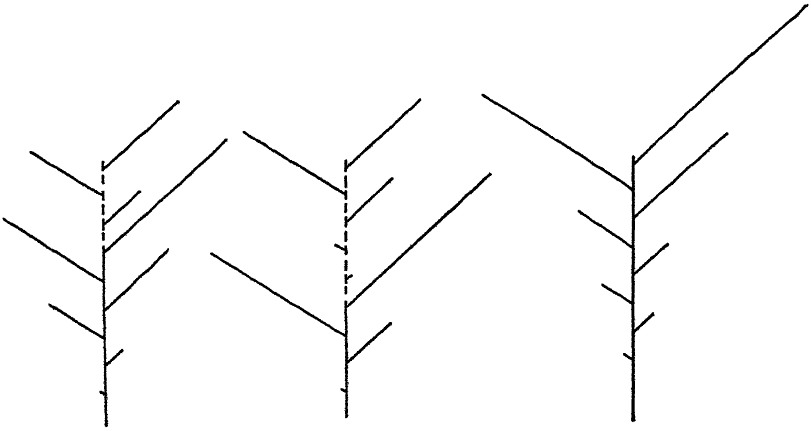

Fig. 2. Chinese lemon cuttings. Comparative length and distribution of sprouts. Dotted line represents the part of the cutting which was taped.

Further evidence that we are here dealing with two physiological units resides in the fact that only the uppermost buds of each part produced sprouts. In 30 per cent of the cuttings whose upper three buds were taped the third bud remained dormant when the tape was removed; whereas in the other set only 6 per cent of the cuttings produced sprouts from all five buds and as a rule the fourth and fifth bud remained dormant. This was still more marked in another experiment to be mentioned later. The average distribution and length of sprouts on the average cutting is presented diagrammatically in figure 2.

The question arises as to whether or not these observed facts shed any light upon the nature of the polar character of regeneration in stems. There is no doubt that the growth of the cuttings under the conditions of the experiment depends upon the material stored in the stem, and that this supply becomes exhausted regardless of the number or position of the sprouts produced. According to Loeb[16] the sprout or sprouts which grow out first attract all the available material, hence the other buds remain dormant. The above investigation shows clearly that this is not true for Chinese lemon cuttings, because in many cases the sprouts below the inhibited portion were of considerable length before the tape was removed and yet growth was not prevented in the apical region.

The interpretation of the results of earlier experiments[20] was based on the assumption that an inhibitory substance, produced by the growing apical sprouts, passed toward the basal part of the cutting and thus inhibited the development of sprouts in that region.

From this experiment, it is obvious that that assumption was inadequate, because in this case the apical region of the cuttings had no sprouts to produce an inhibitory substance and, furthermore, when apical sprouts were produced, they were unable to suppress the growth of subapical sprouts.

If we assume that dominance is due to an axial gradient of metabolism declining steadily with the distance from the apex, then a lemon shoot ought to produce a gradation of sprouts from apex to base. This gradation however appears only in a part of the shoot. Furthermore, less growth ought to be produced in the subapical than in the apical region. This was not the case with taped cuttings, where both the upper and lower portions produced growth in proportion to their mass. Table 3 also shows that there is no consistent difference in the weight or length of sprouts produced by the different parts of the mother shoots.

A possible explanation of these experiments is based upon the view held by Curtis[5] that some substance necessary for growth passes upward through the phloem, On this assumption, in the control cuttings the growth promoting substance would move upward until it reaches the uppermost bud or buds which are in condition to make use of it.

But cuttings planted upside down also produce sprouts from the apex, hence we cannot say that this substance can move only upward. We have seen that in the free portion of the taped cuttings sprout growth started later than in the apical portion of the control cuttings. This time factor is significant. We may assume that the transformation of food reserves into growth-promoting substances is a gradual process which begins at the apex. This view is strengthened by the fact that when only the three uppermost buds were inhibited, the delay in the outgrowth of buds immediately below the tape was not so great as when the five uppermost buds were inhibited.

No definite reason can be given as to why the transformation of food reserves into growth-promoting substances should begin at the apex. Evidently it takes place just as quickly in taped as in untaped cuttings for sprouts will break through weak places in the tape before there is any sign of growth below the tape.

The results obtained warrant the assumption that the earlier release from dormancy of the buds in the apical region is due to the gradual transformation of food reserves into growth-promoting substances from apex to base. The dormancy of subapical buds may be assumed to be due to the ability of the actively growing apical sprouts to draw on the entire supply of growth-promoting substances as fast as they are formed. If the growth of apical sprouts were dependent solely upon the supply of these substances which is present in that region, then buds all along the cutting would have to grow out in order to account for the mass relation obtained.

It is evident from table 4 that the growing sprouts below the taped portion cannot draw on the supply of growth-promoting substances which are stored up in the apical part.

It seems to the writer that the above explanation is more plausible than that based on the downward flow of some inhibitor. A recent investigation by Gardner[6] also indicates that nutritive factors are involved in the initiation of sprout growth.

(b) UNDETACHED EUREKA LEMON SHOOTS

Before proceeding with this discussion, it will be profitable to see whether or not the foregoing concept can also be applied to the behavior of attached shoots under orchard conditions. For this purpose the following experiment was made. At the beginning of the growing season, upright, unbranched, one-year-old Eureka lemon shoots were cut back to stubs 50 cm. in length. This left about 24 buds on each shoot. On one set of 28 shoots one-fourth of the uppermost buds were wrapped with rubber tape and on another set of 28 shoots, one-half were so treated. As in the case of the cuttings, sprouts appeared from the free portion. On a certain number of shoots in each set the tape was removed when at least one of these sprouts reached a length of 5, 15, and 30 to 40 cm., respectively. Twenty untreated shoots served as a control.

Measurements of the length of each sprout produced were made weekly throughout the growing season. A few shoots were taped to the last bud, the intention being to release the buds one by one as sprouts developed.

The results obtained are similar to those obtained with Chinese lemon cuttings. Tables 5 and 6 show that in no case was growth of the free region suppressed after unwrapping the apical portion. Figures 3, 4, 5, and 6 are representative graphs of the growth curves of the free and taped portion. They show with one exception that the growth curves of the two portions run approximately parallel. This exception occurred with shoots whose upper one-half was freed when at least one subapical sprout was 5 cm. in length. Here the growth of the apical portion slightly exceeded that of the subapical one, but not until after the latter had completed its first growth cycle. On the average the control shoots produced sprouts from the uppermost seven buds. The taping of one-fourth of the shoot included from five to seven buds, hence some of the buds below the tape would have produced sprouts without inhibition of the apical portion. But when the taping involved one-half of the shoot, which included from ten to twelve buds, the subapical sprouts were outside of the normally active region.

In discussing the behavior of Chinese lemon cuttings it was emphasized that the difference in the length of time required for apical and subapical buds to produce sprouts may be a factor in regeneration. This time factor was very much more marked in lemon shoots. The subapical sprouts of the shoots whose upper half was taped, appeared one to two weeks later than the sprouts on the control shoots. In the other set a delay of two or three days was noticed. The importance of the position of the bud was still more marked when the taping extended to the last bud. In this case it was at least three weeks before a sprout appeared; besides, these sprouts were weak and yellow. No delay in the appearance of the sprouts was observed on shoots which were cut back to 10, 15 or 25 cm.

TABLE 5

EUREKA LEMON SHOOTS; SHOWING THE EFFECT OF TAPING THE UPPER FOURTH

Shoot No.	Total length of subapical sprouts		Total length of apical sprouts, cm.
	When apex was freed, cm.	Final length, cm.	
21	18	363	401
10	19	132	210
23	20	291	106
7	21	152	561
19	22	223	133
16	22	344	175
4	23	678	208
22	24	392	156
12	25	267	152
28	27	213	75
17	30	104	70
25	35	340	48
3	45	199	116
6	49	412	282
11	53	211	65
15	56	179	141
13	60	70	128
5	66	122	135
26	69	314	269
27	70	289	93
18	121	317	256
8	142	449	237
14	149	268	118
9	157	395	266
1	161	356	274
20	161	281	101
2	200	387	42

TABLE 6

EUREKA LEMON SHOOTS; SHOWING THE EFFECT OF TAPING THE UPPER HALF

Shoot No.	Total length of subapical sprouts		Total length of apical sprouts, cm.
	When apex was freed, cm.	Final length, cm.	
9	7	154	306
15	7	72	274
18	9	135	202
11	16	60	113
3	16	223	276
1	19	260	163
10	19	130	151
20	23	566	306
13	28	205	188
5	33	218	141
23	33	487	186
14	33	242	178
2	34	201	159
27	45	239	247
26	48	96	251
25	49	222	271
19	54	137	264
7	62	281	130
16	70	136	83
17	81	309	207
21	83	88	74
28	98	411	462
24	101	400	145

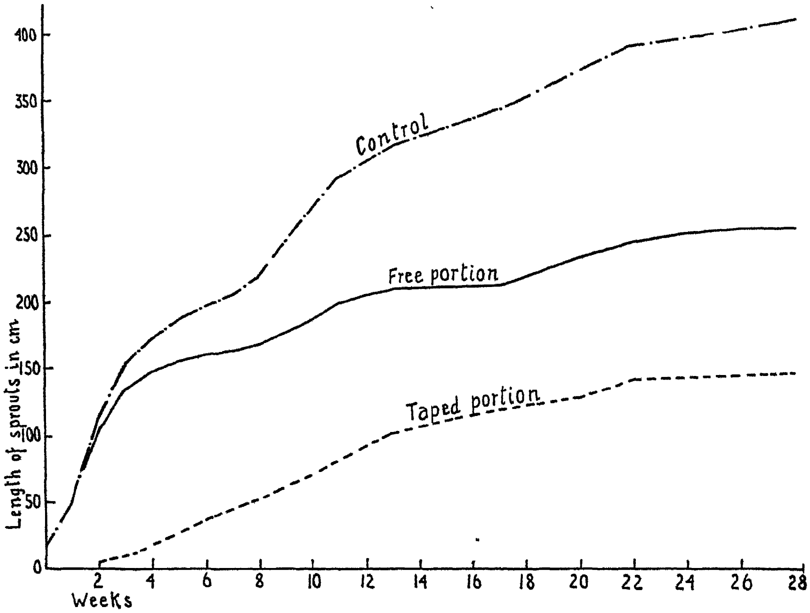

Fig. 3. Vertical Eureka lemon shoots. Average total sprout growth per shoot. Upper fourth taped; freed when at least one subapical sprout was 5 cm. long. Control—not taped.

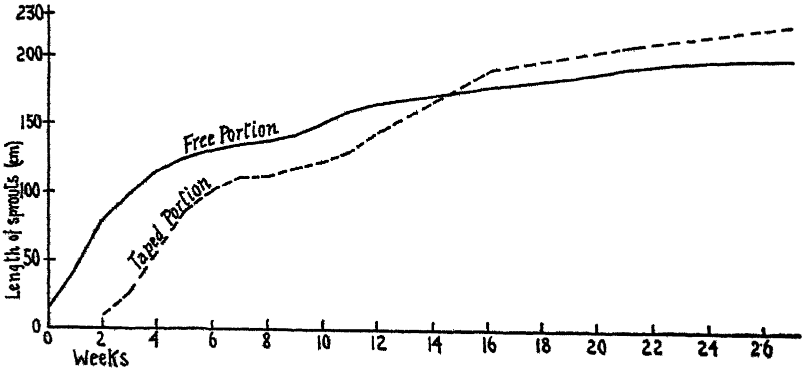

Fig. 5. Vertical Eureka lemon shoots. Average total sprout growth per shoot. Upper half taped; freed when at least one subapical sprout was 5 cm. long.

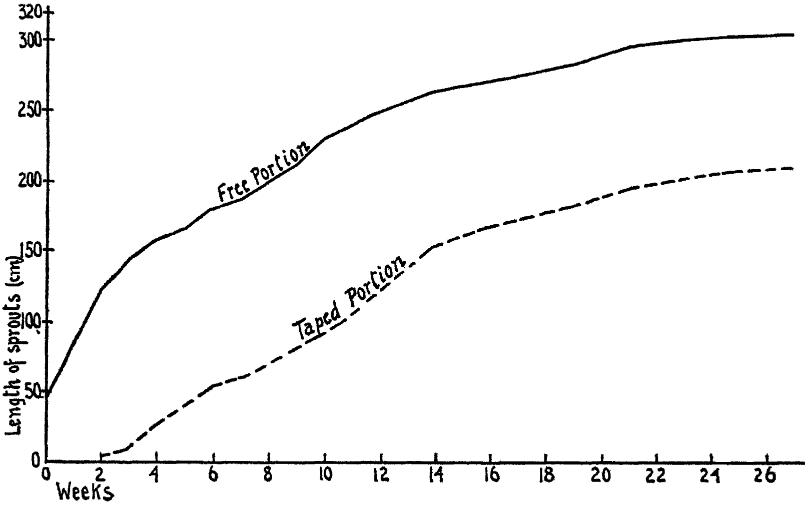

Fig. 4. Vertical Eureka lemon shoots: Average total sprout growth per shoot. Upper fourth taped; freed when at least one subapical sprout was 15 cm. long.

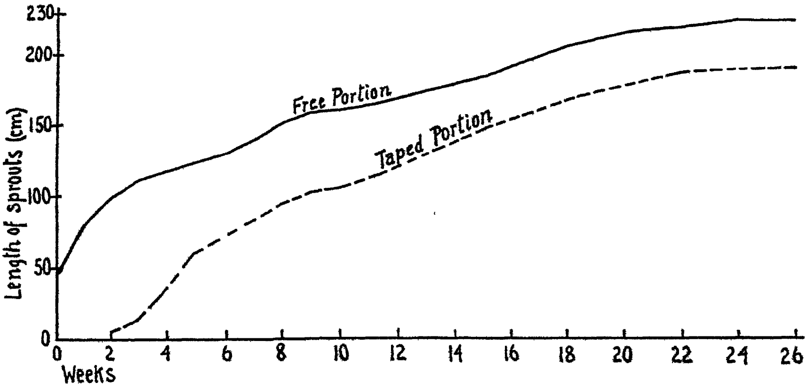

Fig. 6. Vertical Eureka lemon shoots. Average total sprout growth per shoot. Upper half taped; freed when at least one subapical sprout was 15 cm. long.

An important fact, observed also with Chinese lemon cuttings, was the dormancy of certain buds which under normal conditions would have grown out. Figure 7 showes diagrammatically the average length and distribution of the sprouts on the three sets of mother shoots. It is seen that after the freeing of the upper five taped buds, only three grew out, while nine sprouts were produced by the lower untaped portion. In the other set only six out of ten buds grew out in the upper portion while nine sprouts were produced by the lower portion. The average number of sprouts produced was twelve per shoot in the former taped set, fifteen in the latter, and eight in the controls. The difference in the number of sprouts or in their size previous to unwrapping the apex had practically no effect upon the total amount of growth (table 7).

TABLE 7

EUREKA LEMON SHOOTS; SHOWING THE EFFECT OF TAPING THE UPPER FOURTH AND UPPER HALF

Length in cm. per 100 sq. cm. of bark area of shoot.

Upper fourth of mother shoot taped

Average total length of sprouts produced by			
Free portion		Apical portion (final length)	Entire mother shoot
When tape was removed	Final length		
18	186	325	221
29	197	406	249
97	158	371	251
Upper half of mother shoot taped			
15	200	224	213
42	220	187	203
67	255	200	228
Control			
....	....	.. ..	203

In order to test the reliability of these results the following experiment was made. At the beginning of the growing season 14 pairs of uniform unbranched, one-year-old shoots, situated similarly on the trees, were cut back to the mature wood. On one shoot of each pair, notches were made in the bark just above many of the subapical buds, in order to force out a greater number of sprouts. The shoots thus treated produced about twice as many sprouts as the untreated shoots. At the end of the growing season the sprouts on both sets of shoots were measured and their dry weight determined. The results are summarized below. The figures are based on 100 sq. cm. of shoot area.

	Total length of sprouts	Dry weight of sprouts
Notched	207 cm.	30 grams
Control	219 cm.	35 grams

The ratio between dry weight of mother shoots and dry weight of sprouts produced was found to be as 1 to 1.8 for each case.

The evidence for vertical lemon shoots is fairly conclusive that the amount of growth produced is approximately proportional to the size of the mother shoot regardless of the number or distribution of the sprouts. It may be mentioned here that a similar relation exists in horizontal shoots.

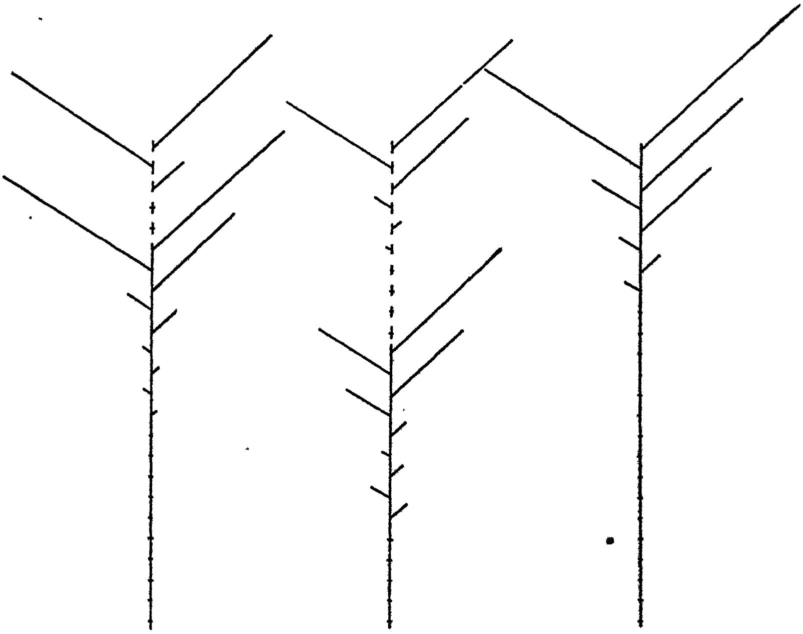

Fig. 7. Vertical Eureka lemon shoots. Comparative length and distribution of sprouts. Dotted line represents the part of the shoot which was taped.

(*c*) DISCUSSION

The results obtained with lemon shoots corroborate in a general way those obtained with Chinese lemon cuttings. When the outgrowth of buds in the upper one-fourth or one-half is prevented, the parts behave as separate physiological units. A few remarks concerning the cyclic growth of lemon sprouts may be made. Normally not more than three cycles are made by the sprouts nearest the apex; those further down usually make only one or two cycles. The growth made during the greater part of the first cycle probably depends entirely upon the reserve material stored in the shoot. For the first few weeks all sprouts grow at approximately an equal rate, then the apical ones gain the ascendency. During the second and third cycles elongation is practically confined to a few apical sprouts.

For the purpose of our discussion we may divide the seasonal growth into two periods, one during which growth probably depends on the stored material and the other during which nutrients are supplied. The first period, during which sprout growth is initiated, is the phase with which we are mainly concerned at present.

In the shoots the transformation of food reserves into growth-promoting substances is probably a gradual process which begins at the apex. As is the case of cuttings, the growing apical sprouts appropriate the supply of growth-promoting substances as fast as it is formed, hence the buds farther down remain dormant.

The fact that the time required for subapical sprouts to grow out is in proportion to the length of the portion which is taped, suggests the possibility that the formation of growth-promoting substances begins at the apex and in the cuttings this formation takes place whether or not the upper portion is taped. Evidently the growing subapical sprouts can draw on the supply of growth-promoting substances only from below the point of origin of the sprout.

During the second period only a few apical sprouts continue to elongate while the others below make no further growth. The amount of raw materials furnished each shoot depends upon the size of the root system and the number of growing tips. Hence it is conceivable that under favorable conditions each growing tip receives its proportion of this material. But this does not explain why it should only benefit a few of the uppermost sprouts.

A possible explanation is that during the second period the rapidly growing sprouts exert an inhibiting influence upon the growth of the subapical sprouts. This influence, as Loeb's[14] results suggested, may be due to an actual substance (a hormone) which is produced in the growing tip and which, as it travels downward, prevents further elongation of subapical sprouts. Reed[17] found evidence in pruned pear shoots which he considered a confirmation of Loeb's inhibitory hypothesis. In subsequent studies Reed[18, 19] applied this concept to explain the behavior of apricot shoots and of organisms in general.

Barker and Lees,[1] working with pear shoots, came to the conclusion that in addition to the action of an inhibitor other factors such as temperature, bud strength, root action and variety influences determine the growth of buds on the mother shoot.

A general objection to the inhibitor theory is that raised by Harvey.[10] He and others argue that if such a substance is produced in the dominant region, growth should be depressed there also because it would be in greatest concentration there. This, however, would not be true if the inhibiting substance is carried away as fast as it is formed. Since nothing is known about the nature of this substance the objection cannot be met unless we accept Robertson's hypothesis.[21]

It is the writer's view that the apical dominance in attached lemon shoots is governed by two distinct processes. During the initial period the release from dormancy of the buds and sprout growth is governed by growth-promoting substances. Later apical dominance may be ascribed to an inhibiting substance which is produced by the rapidly growing apical sprouts and influence their subapical neighbors. In dealing with cuttings we are concerned only with the release from dormancy.

IV. EFFECT OF INJECTIONS OF VARIOUS CHEMICAL COMPOUNDS ON THE OUTGROWTH OF BUDS

In the preceding part of this paper the view was expressed that the upward movement of growth-promoting substances contained within the cuttings, together with a time factor, are concerned in determining the apical dominance in Chinese lemon cuttings. The next step was to study the effect of substances introduced into the cuttings on the order of bud development and the amount of growth.

Curtis[4] found that treatment with potassium permanganate results in a very marked increase in root growth of various woody cuttings. He also found that immature cuttings produced better root growth when treated with cane sugar. In maturer cuttings this treatment generally resulted in increasing the top growth and in the development of subapical buds which normally remain dormant.

Gardner[6] succeeded in bringing about the outgrowth of basal buds of pear cuttings by introducing a five-tenths per cent solution of sodium nitrate into the basal part of the cutting. Gardner considers it probable that the absorption of sodium nitrate by the basal portion of the cutting lowered the carbohydrate-nitrogen ratio by increasing the nitrogen content.

(a) LIGUSTRUM CUTTINGS

The material was obtained during the winter from a hedge growing in Riverside, California. This plant is an evergreen but goes into dormancy about the same time as the Chinese lemon. Twenty cuttings, each possessing five nodes taken from mature shoots, were used for each treatment, which consisted in the introduction of a solution of a chemical. In ten of them the solution was drawn from base to apex and in the other ten from apex to base. The solution was drawn through the cuttings by means of a filter pump. The cuttings were removed after at least several cubic centimeters of the solution had collected above. They were then thoroughly rinsed with water, and the part which stood in the solution and to which the tubing was attached was cut off. The cuttings were planted vertically in flats containing river sand and kept in a basement room in which a temperature of 25° to 27° C. and a humidity of about 75 per cent were maintained. Light was furnished by a 200-Watt Mazda daylight bulb placed about one meter from the cuttings. Evidently the amount of light was insufficient as none of the cuttings produced roots. Some cuttings treated similarly and exposed to sunlight produced roots but the twig growth was similar to that made in the culture room. Table 8 gives the summary of the results. The manner in which the solution was drawn through the cutting had no effect upon growth, hence no separate data are given.

The important point is that none of the compounds used stimulated greater growth than distilled water. It is remarkable that the acids and bases did not injure the cuttings to a greater extent. It will be noticed that some cuttings died in every lot. Treated with M/5 $Ca(NO_3)_2$ only two cuttings failed to grow while, with the weaker solution sixteen died and the growth was also poor. It is worthy of remark that in most cases more cuttings died with the weak than with the strong solutions. The reason is not evident.

It was observed that in no case was the order of bud development reversed. The greatest deviation from the normal was found in cuttings which received M/5 $Ca(NO_3)_2$ solution. The fact that this compound did not increase growth would indicate that it interfered with the upward movement of growth-promoting substances in the uppermost buds.

Since no roots were produced, Curtis' findings in regard to the root-stimulating effect of potassium permanganate could not be verified. Under the conditions of the experiment both concentrations gave poor top growth. A few cuttings treated with this compound and placed in sunlight did not give better results than those treated with sugar or distilled water.

TABLE 8

LAGUSTRUM CUTTINGS INJECTED WITH VARIOUS SOLUTIONS, AND GROWN IN SAND

Treatment	Number of cuttings		Total average length of twigs per cutting in cm.	Order of best growth	Average number of nodes which grew
	Dead	Growing			
M/5 HCl	4	16	8.9	7	2.1
M/25 HCl	10	10	7.0	11	1.5
M/5 KOH	8	12	7 3	10	1 6
M/25 KOH	7	14	7.7	9	1.7
M/5 NaOH	8	12	6.3	14	1.3
M/25 NaOH	8	12	5.8	15	1 4
M/1 dextrose	3	17	10.7	3	1 5
M/5 dextrose	10	10	8 8	8	1.5
M/10 $KMnO_4$	7	13	7.3	10	1 9
M/25 $KMnO_4$	5	15	6 4	13	1 5
M/5 $Ca(NO_3)_2$	2	18	10.5	4	2.4
M/25 $Ca(NO_3)_2$	16	4	6.5	12	1.4
M/5 $NaNO_3$	9	11	10 3	5	1.7
M/25 $NaNO_3$	10	10	9.1	6	1.8
Ether water	8	12	11.5	1	2.0
Distilled water	7	14	11.4	2	1.6
Control	9	11	9.1	6	1.4

(*b*) MATURE CHINESE LEMON CUTTINGS

Cuttings of this plant grow well under the conditions described above. In a preliminary experiment injected cuttings of uniform size were suspended in saturated air. For each treatment 14 cuttings were used. The following compounds were used in various concentrations: HCl, KOH, NaOH, dextrose, ether-water and distilled water.

None of these solutions had any influence on the order of bud development. The average total length of sprouts produced per cutting is given below. None of the solutions omitted gave better results.

M/25 HCl	5.7 cm.	Ether water	3.0 cm.
M/25 NaOH	5.3 cm.	Distilled water	9.7 cm.
M/25 KOH	4.4 cm.	Control	5.3 cm.
M/1 dextrose	5.7 cm.		

There is no evidence of any effect of any of the injections, except an increase of growth with distilled water, a decrease with ether-water and a probable slight decrease with KOH. There was no deviation from the normal order of bud development.

In another experiment cuttings possessing 7 to 11 buds were made and well sampled so that each lot contained parts of several trees. For each treatment 15 cuttings were used. The method of injecting the solutions and the condition under which the cuttings were grown were the same as those previously described except that the cuttings were placed in sand. Before planting, the green weight and bark area of each cutting were determined. In all cases twig growth ceased after the third week. After the fourth week the twigs and roots were cut off, weighed and measured.

TABLE 9

CHINESE LEMON CUTTINGS INJECTED WITH VARIOUS SOLUTIONS

Green weight of sprouts and roots in grams per 100 grams of green weight of cuttings.

Number of cuttings dead	Treatment	Weight of sprouts	Order of greatest sprout growth	Weight of roots	Order of greatest root growth
1	M/5 HCl	23.1±1.25	3	4.1±.54	8
0	M/25 HCl	22.9±1.06	4	4.1±.50	8
2	M/5 NaOH	15.5±1.27	14	1.6±.35	12
1	M/25 NaOH	28.2±1.10	1	6.0±.59	6
3	M/5 LiNO$_3$	16.6±2.13	13	3.7±.51	9
0	M/25 LiNO$_3$	24.8±1.13	2	5.9±.45	7
0	M/5 Ca(NO$_3$)$_2$	18.7±1.06	10	6.2±.34	5
1	M/25 Ca(NO$_3$)$_2$	20.3±1.04	9	6.6±.48	3
0	M/1 dextrose	20.3± .95	9	6.7±.44	2
0	M/5 dextrose	21.0± .92	7	6.7±.70	2
0	M/5 NaNO$_3$	21.4±1.24	6	8.5±.79	1
0	M/25 NaNO$_3$	20.7± .74	8	6.4±.50	4
0	Distilled H$_2$O	22.6± .88	5	8.5±.69	1
0	No treatment	18.2±1.36	11	6.7±.45	2
5	Hoagland solution, no Fe	6.1± .95	20	0.9±.26	15
7	Hoagland solution+Fe	6.4± .59	19	0.5±.17	17
4	Fe tartrate	7.9±1.16	17	1.9±.46	11
9	M/25 KMnO$_4$	5.3± .72	22	0.6±.24	16
4	M/5 K$_2$HPO$_4$	3.1± .49	23	0.0	18
4	M/25 K$_2$HPO$_4$	6.8± .75	18	1.3±.33	14
3	M/5 citric acid	5.7± .52	21	.5±.18	17
3	M/25 citric acid	7.9± .76	17	1.4±.24	13
3	M/5 (NH$_4$)$_2$SO$_4$	8.5± .85	16	1.3±.27	14
4	M/25 glycocoll	11.5±1.36	15	2.2±.35	10

It appears from the data given in table 9 that none of the solutions used caused significantly greater total growth than distilled water. $M/5 LiNO_3$ was the only solution which caused visible injury to the cuttings. In this case one or two centimeters of the apex turned brown within a few days. The injury was probably caused by a high concentration of the salt as a result of the evaporation of water from the cut surface.

The treatment with HCl did not affect top growth and only slightly reduced root growth. The sprout growth on cuttings treated with M/5 NaOH was poor, compared with that on cuttings treated with M/25 NaOH. Cross sections made from cuttings treated with the stronger solution of NaOH showed reddish discolorations in the woody part of the cutting through which the solution passed. Within these areas starch was present while none was found in the unaffected regions.

All of the organic compounds used, with the exception of dextrose, retarded growth. There is no indication that dextrose increased either top or root growth. It is of interest to note that Hoagland solution retarded growth although one of its constituents is $Ca(NO_3)_2$ which gave fair results. It is possible that the presence of K_2HPO_4 in Hoagland solution may be the retarding factor since when it was used alone very little growth was produced.

The fact that with some of the compounds the growth produced was poor may be due to interference with the movement of growth-promoting substances. There was some indication that certain compounds hastened twig growth, but the total amount of growth was not increased. None of the substances used had any effect upon the order of bud development.

(*c*) IMMATURE CHINESE LEMON CUTTINGS TREATED WITH SUGAR SOLUTIONS

Cuttings from which all leaves were removed were taken from the apical part of immature shoots on May 23. Each cutting possessed seven nodes. They were divided into six lots of 18 cuttings each. The solutions used were M/5 and M/10 dextrose, M/5 and M/10 cane sugar and distilled water. One lot served as a control. The cuttings of each lot stood in the solution to a depth of one centimeter for 24 hours without the application of suction. They were then rinsed with distilled water and planted in flats containing river sand. On July 29 the cuttings were removed and the roots and sprouts weighed. The results are summarized in table 10.

The amount of sprouts produced by cuttings treated with distilled water and by the untreated ones was far in excess of that produced by cuttings treated with the various sugar solutions. The amount of roots produced by the cuttings treated with M/10 cane sugar solution compares favorably with that produced by the control cuttings. It will be seen that with all sugar solutions the percentage of the cuttings which died is considerable while none of those treated with distilled water or of the controls died. It is therefore probable that leaving such cuttings in the sugar solutions longer than 24 hours would result in a still greater loss.

TABLE 10

IMMATURE CHINESE LEMON CUTTINGS, MAY, 1924; 18 CUTTINGS IN EACH LOT

Treatment	Average total length of sprouts per cutting, cm.	Average green weight (grams)		Number of cuttings dead
		Sprouts	Roots	
M/5 dextrose	2.3	28	.12	6
M/10 dextrose	2.1	.22	.10	7
M/5 cane sugar	1.5	.21	.08	7
M/10 cane sugar	2.6	.44	.19	7
Distilled H_2O	3.4	.66	.15	0
Control	3.8	.74	.21	0

Several cuttings similar to those which were treated were examined for starch. In the apical and middle regions, traces of starch were present in the cambium; in the basal part of the cutting starch was also found in the medullary rays.

None of the treatments had the slightest effect upon the order of sprout development. The sprouts from the first and second bud were always the longest and when the third or fourth bud grew out the resulting sprout was very short. There was practically no difference in the number of sprouts produced.

(*d*) DISCUSSION

The results of these experiments show no effect on the order of sprout development or growth on either mature or immature cuttings with any of the substances injected. In the case of M/5 NaOH reduced growth was probably due to the locking up of a part of the food reserve supply. However the injected cuttings started to grow just as quickly as others which produced a greater amount of growth.

The detrimental effect of some of the compounds upon mature cuttings may be explained on the assumption that these compounds interfered with the transformation of food reserves into growth-promoting substances. This view is strengthened by the fact that the release from dormancy of the buds was delayed or entirely prevented. The fact that many of them remained alive for many weeks shows that these compounds as such were not otherwise injurious.

The retarding effect of Hoagland solution is of interest. It contains both $Ca(NO_3)_2$, which is not detrimental, and K_2HPO_4, which is detrimental to the production of sprouts.

V. BEHAVIOR OF HORIZONTAL LEMON SHOOTS

This part of the investigation is an attempt to study the factors involved in the distribution of sprouts on horizontal shoots and cuttings.

All material was obtained from trees growing on the grounds of the Citrus Experiment Station, Riverside, California. The experiments in the orchard were carried out with shoots of the Eureka lemon and those in the laboratory with Chinese lemon cuttings.

With upright lemon shoots are cut back to the mature wood and brought into a horizontal position, sprout development will be confined to the dorsal side with the exception of one or two sprouts which will appear on the ventral side close to the cut. Cuttings of the Chinese lemon grown in a horizontal position behave similarly.

It is often claimed that by bending a shoot into a horizontal position the tissues at the bend are compressed and thus impede the flow of sap to the buds on the ventral side. This view is not supported by the facts. In the first place no tissues are compressed when cuttings are grown horizontally and yet sprouts have not been seen to occur on the ventral side. Secondly, lemon shoots always exhibit this behavior whether the tissues are compressed or stretched. A long shoot bent into a U-shape and tied so that both arms lie in the same plane develops sprouts only on the dorsal side of each arm. Figure 8 shows two Chinese lemon plants both of which were bent in the same way. One plant was grown upside down so that compressed tissues were on the dorsal side and the other plant remained in the normal position. It will be seen that in both cases growth was distributed along the dorsal side.

Light is apparently not a factor of primary importance in initiating sprout growth of lemon shoots or cuttings. Chinese lemon cuttings suspended horizontally in saturated air produced sprouts only on the dorsal side in light as well as in darkness. This is also true when the dorsal side is darkened and the ventral side illuminated.

Loeb[15] suggested that the sprouts on the dorsal side of a horizontal shoot may inhibit growth on the ventral side. He was successful in inducing outgrowth on the ventral side of a horizontally suspended stem of *Bryophyllum calycinum* when he removed the buds on the dorsal side. No evidence of this inhibiting action could be obtained with lemon shoots. Cuttings were split longitudinally and either suspended in saturated air or placed in moss or sand. Whenever the halves of the cuttings were placed so that the buds were on the ventral side, no growth occurred, but when they were placed in the reverse position sprouts developed promptly. It was observed that the growth from a cutting divided in this manner was weaker than that made by whole stems.

Destroying the buds on the dorsal side of either cuttings or horizontal shoots had no effect on the buds of the ventral side but as a result of this operation many cuttings and shoots produced sprouts from adventitious buds on the dorsal side.

On several horizontal shoots no dorsal sprouts were allowed to grow for three years, yet none of the buds on the ventral side, with the exception of one or two close to the apex, showed any signs of growth.

The influence of position upon the development of sprouts can be shown in other ways. When a horizontal cutting is allowed to develop sprouts from the dorsal side and is then revolved through an arc of 180 degrees, the buds from the previously ventral side then produce sprouts while the original sprouts gradually cease growing and in some cases die.

In order to study this behavior quantitatively the following experiment was made. Forty one-year-old vertical lemon shoots were cut back to the mature wood and bent into a horizontal position before growth started in the spring. Twenty of these shoots remained in this position during the entire season, while the twenty others were bent in the opposite direction four weeks after the appearance of sprouts when the average length of the sprouts was 5.4 cm.

In less than two weeks the buds which were previously on the ventral side became active and by the end of the growing season the average length of sprouts per mother shoot was 15.1 cm., as compared with 12.6 cm. for the sprouts which were originally on the dorsal side of the reflexed shoots. This shows that though the original sprouts were not suppressed by the new set the latter exceeded the former in spite of a later start.

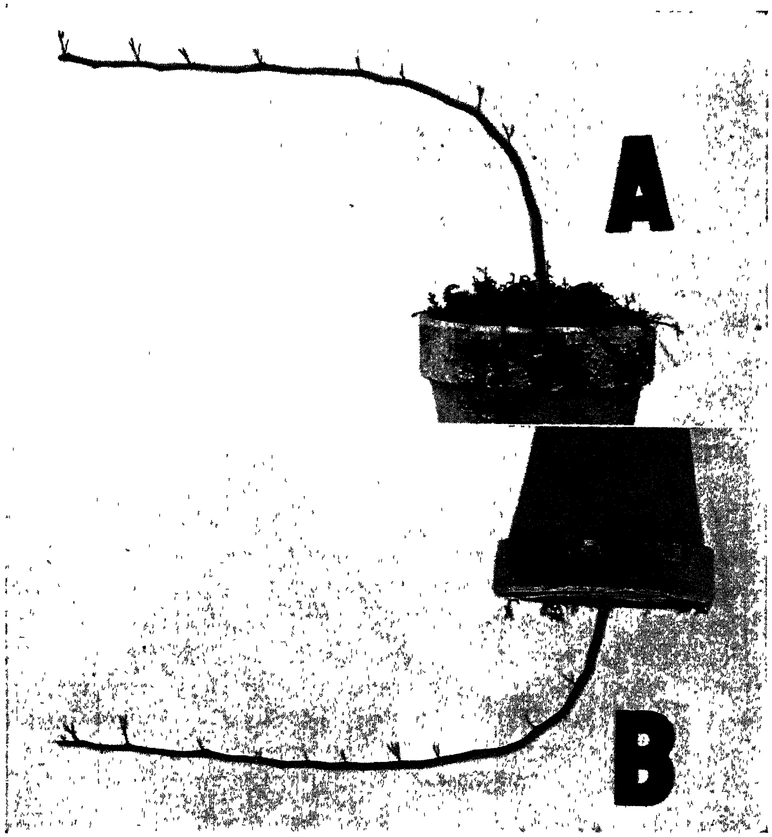

Fig. 8. Chinese lemon shoots bent into a horizontal position. (*a*) Right side up; (*b*) upside down.

The average length of sprouts produced by the reflexed shoots, 27.7 cm., compares favorably with that produced by horizontal control shoots, which was 24.6 cm. In total growth there is practically no difference between the two sets of shoots. In centimeters of length of sprouts per 100 sq. cm. of shoot area, the reflexed shoots produced 66 cm. and the control 76 cm. The average number of sprouts on the former shoots was twelve and on the latter seven. In other words, the total amount of growth is not much influenced by the number of sprouts produced. The only obvious difference was that where fewer sprouts were produced their length was greater.

It has already been said that the seasonal growth of lemon sprouts is made in three cycles. The growth on 225 horizontal mother shoots as determined by measuring the total increments of all sprouts was as follows: 1st cycle, 181 cm., 2nd cycle, 77 cm., and 3rd cycle, 71 cm.

It will be remembered that the original set of sprouts averaged 5.4 cm. when the position of the shoot was changed, and that they reached an average length of 12.6 cm. This corresponds to the average length of 11.5 cm. of sprouts produced by the control shoots during the 1st cycle. It is evident that the change in position did not influence the growth of the down-pointing sprouts until the completion of the 1st cycle, while the sprouts on the dorsal side continued their growth to the end of the season.

These results suggest that gravity may be one of the factors responsible for the distribution of growth on horizontal shoots. For the purpose of studying the effect of gravity the following experiments were made.

Several dormant Chinese lemon cuttings were suspended horizontally in saturated air and revolved through an arc of 180 degrees once every week. Sprouts appeared on both sides within ten days and the rate of growth was approximately the same.

Another set of dormant Chinese lemon cuttings were revolved continuously in a moist atmosphere. This resulted in growth from all sides, and the sprouts were confined to the apical portion of the cutting. When the motor was stopped for about two weeks the sprouts which happened to be on the dorsal side continued to elongate; if kept in this position long enough some of the sprouts pointing downward deteriorated.

From these observations it seems that gravity is a controlling factor in the distribution of sprout growth. The cuttings revolved through an arc of 180 degrees once every week produced sprouts which were in the same plane. By revolving the cuttings continuously the force of gravity was equalized and consequently growth occurred on all sides.

(*a*) EFFECT OF NOTCHING ON OUTGROWTH OF BUDS

Unbranched dormant lemon shoots were cut back to the mature wood and tied in a horizontal position. From two to four buds on either the dorsal or the ventral side of each shoot were treated in the manner described below. For each treatment twenty-five shoots were used.

1. A slanting cut was made, beginning about 1 cm. above the bud and extending at an angle of about 20 degrees into the wood to a point 1 cm. below the bud, thus severing the bud from the mother shoot, except on the lower side. A piece of mica was inserted to keep the cut from healing over.
2. A cut was made as above but beginning below the bud.
3. A notch was made above the bud, removing a small crescent-shaped piece of bark.
4. A notch was made as in the last case but below the bud.

The results are given in table 11. It is evident that buds grow if a notch or cut is made above the bud but that sprout growth is inhibited when a notch or cut is made below.

TABLE 11

HORIZONTAL LEMON SHOOTS; SHOWING THE EFFECT OF NOTCHING UPON SPROUT GROWTH

Treatment	Number of buds treated	Per cent of buds which grew	Average total length of sprouts (cm.) per 100 sq. cm. of shoot area
Dorsal buds:			
1	70	98.6	103
2	68	1.5	96
3	71	91.6	111
4	61	9.8	116
Ventral buds:			
1	80	73.3	104
2	71	0.0	109
3	70	51.4	112
4	77	1.3	101
Controls	....	....	106

In order to determine whether the age of the bud on the mother shoot is a factor, buds of the same age were inserted on both dorsal and ventral sides of shoots. These inserted buds were notched in the manner previously described and the results were identical. An attempt was also made to force out buds on the ventral side of the shoot by one longitudinal notch made on each side of the bud; but the results were negative.

It was observed that the twigs produced on the ventral side as a result of notching made the first cycle of growth only. Furthermore several attempts to induce twig growth on the ventral side by notching or cutting later in the season failed.

In the discussion of vertical shoots data were presented to show that the amount of growth produced depends upon the size of the mother shoot. The last column in table 11 shows that this also holds for horizontal shoots.

Although these values agree closely among themselves yet they are only about one-half as large as those obtained with vertical shoots (table 6). But the two cases are entirely different. For the vertical shoots the values given represent the entire shoot whereas in the horizontal shoots only a portion of the entire shoot is represented. In bending a lemon shoot, only about one-half of it can be brought into a horizontal position while the other part remains more or less in a vertical position. This latter portion of the shoot produces the most vigorous growth especially on the convex side of the bend. The growth of these sprouts if left undisturbed retards the development of the sprouts on the horizontal part of the shoot.

Dormant Chinese lemon cuttings contain an abundance of starch but the few sprouts produced on the dorsal side, when grown in a horizontal position, apparently are able to utilize it since the starch has disappeared entirely when the sprouts have reached their maximum length. Sprouts could not be forced out on the ventral side by preventing sprout growth on the dorsal side. If, however, on such shoots a few of the buds on the ventral side were notched above, growth took place from these buds provided the notching was done early in the season.

(*b*) PHYSICAL CHARACTERISTICS OF SAP FROM DORSAL AND VENTRAL BARK

Before the beginning of the growing season succulent, one-year-old lemon shoots were cut back to the mature wood and bent into a horizontal position. The horizontal portion of these shoots varied from 50 to 100 cm. in length. In May, when the sprouts on the dorsal side averaged a few centimeters in length, two sets of samples of 7 to 10 shoots were taken. This was repeated once every month throughout the growing season. One set was taken about 2 P.M. and the other before sunrise. The bark of the dorsal and of the ventral sides were separated and thoroughly frozen. The material was then ground up in a meat chopper and the sap expressed with a hand press. The freezing point, the hydrogen-ion concentration and the viscosity of the sap were determined.

For the freezing-point determination the ordinary Beckmann apparatus was used. The depression of the freezing point as read from the thermometer was corrected for under-cooling by the formula given by Harris and Gortner.[9]

The per cent of total solids was determined by means of a refractometer according to the method employed by Gortner and Hoffman.[8] The viscosity was measured with an Ostwald viscosimeter at a temperature of 25° C. The P_H value was determined by comparing samples of sap with standard buffer solutions. The results are summarized in table 12 and presented graphically in figures 9, 10 and 11.

TABLE 12

CHARACTERISTICS OF SAP FROM HORIZONTAL LEMON SHOOTS. UPPER FIGURES FOR EACH DATE PERTAIN TO DORSAL SIDE OF SHOOT AND LOWER FIGURES TO VENTRAL SIDE

Date	Samples taken in afternoon				Samples taken in morning			
	Δ	Total solids (%)	Viscosity ($H_2O=100$) (seconds)	P_H	Δ	Total solids (%)	Viscosity ($H_2O=100$) (seconds)	P_H
May 1	1.224			5.9	1.117			5.8
	1.204			5.8	1.153			5 9
June 6	.982	14 60		5.6	.898	12.50		5.7
	1.005	14.00		5.7	.938	13.10		5.6
July 9	1.067	16.80	190	5.5	.927	15.30	165	5.6
	1.082	16.80	180	5.5	.925	15.30	160	5.7
Aug. 6	1.066	18.40	200	5.5	.918	15.45	170	5.6
	1.056	17.75	195	5 5	889	15.25	170	5.6
Sept. 25	1.208	21.00	230	6.0	1.043	17.65	195	6.1
	1.217	20.40	220	5.9	.999	16.40	175	6.0
Oct. 25	1.252	21.40	310	5.8	1.140	19.60	230	5.8
	1.221	20.80	215	5 8	1.089	18 05	200	5.8

Refractometer and Viscosimeter were not available during the early part of the investigation.

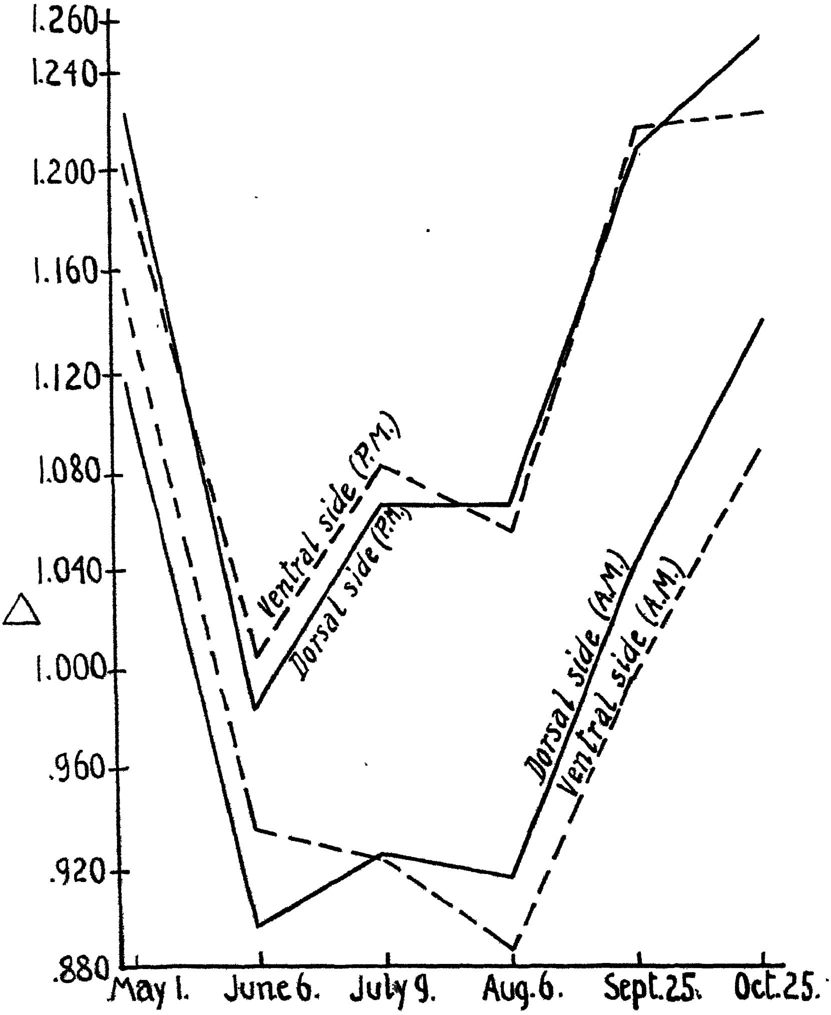

Fig. 9. Horizontal Eureka lemon shoots. Freezing-point depression of sap of dorsal and ventral side.

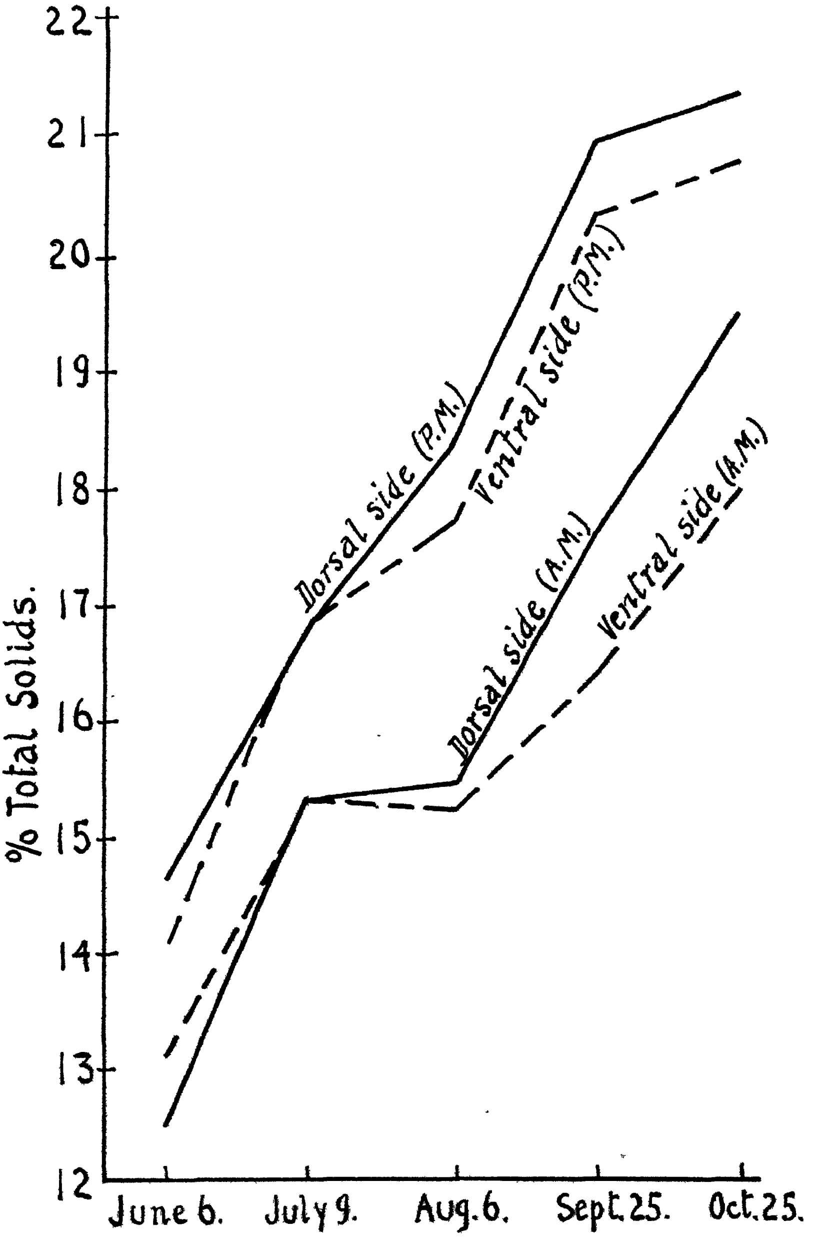

Fig. 10. **Horizontal Eureka lemon shoots.** Per cent of total solids in sap of dorsal and ventral side.

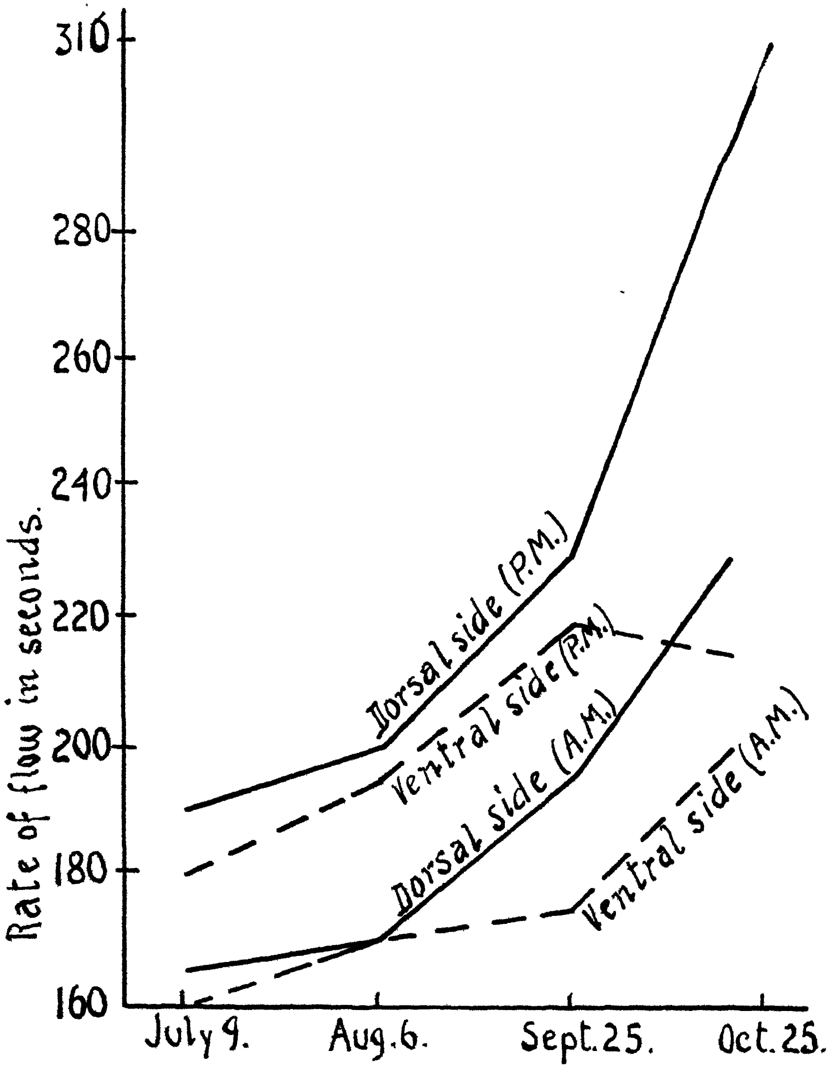

Fig. 11. Horizontal Eureka lemon shoots. Viscosity of sap of dorsal and ventral side compared with rate of flow of water taken as 100.

It is apparent that the freezing-point depression of the sap of the afternoon samples showed practically no difference except in October, when the sap of the ventral side was less concentrated. In the morning samples the ventral side was more concentrated until July, while during the rest of the growing season the reverse is the case.

The curve of total solids follows in a general way that of the freezing-point depression. However, in the case of the afternoon samples, the per cent of total solids is consistently greater on the dorsal side except in July, when the dorsal and ventral sides were alike. The morning samples gave similar results except that the order was reversed during June.

The difference in viscosity between dorsal and ventral sides was small during the early part of the season but it was very marked during October.

The P_H values fluctuated so little that graphs are unnecessary.

The most important point to be considered in the interpretation of these data is, whether any of these methods furnish sufficient evidence that the difference in behavior between the dorsal and the ventral side of horizontal lemon shoots can be related to differences in the sap during the initial period of growth. The determined differences in freezing-point depression and total solids, early in the growing season, were small and inconsistent; we cannot conclude from these data, therefore, that an actual general difference between dorsal and ventral sides existed.

It is interesting to note the great differences in sap concentration between afternoon and morning samples. Sap from leaves and from the bark of the trunk also show this constant difference.

Since none of the data obtained by the various methods mentioned indicate a difference in the sap of dorsal and ventral side early in the season, it was thought that there may be a difference in enzymatic activity. The studies of Heinicke[11] suggest that the catalase activity of apple leaves and bark may be taken as one of the indicators of the nutritive or physiological condition of those tissues. A series of determinations were made in the manner described by Heinicke,[11] with the bark of dorsal and ventral sides of shoots similar to those used for sap studies.

The results are summarized in tables 13 and 14. It will be seen (table 13) that, although the differences are not great, yet they are constant. Until about August 20 the catalase activity was greater in the dorsal than in the ventral side of these shoots. It will be noted that this was true whether the shoots were in full sunlight or in the shade. The samples taken August 25 and September 15 showed a greater catalase activity on the ventral side. This difference in favor of the ventral side becomes very large as the shoots become older (table 14).

TABLE 13

RELATIVE CATALASE ACTIVITY OF DORSAL AND VENTRAL BARK OF LEMON SHOOTS WHICH WERE BENT INTO A HORIZONTAL POSITION IN THE SPRING OF 1924. SAMPLES TAKEN BETWEEN 7:30 AND 10 A.M.

Date (1924)	Position of shoot	Tissue	Seconds required to liberate 1-5 cc. of oxygen				
			1	2	3	4	5
June 25	Full sunlight	Dorsal	10	20	30	60	95
		Ventral	10	20	40	75	125
July 14	Partly shaded	Dorsal	7	15	30	60	95
		Ventral	7	15	35	65	105
July 14	Shaded	Dorsal	7	15	35	60	95
		Ventral	7	20	45	80	120
July 14	Partly shaded	Dorsal	5	12	20	40	65
		Ventral	7	15	30	55	85
July 23	Full sunlight	Dorsal	7	15	30	60	95
		Ventral	7	20	40	80	125
July 23	Partly shaded	Dorsal	10	25	50	90	120
		Ventral	10	25	55	95	135
Aug. 11	Shaded	Dorsal	7	15	30	55	95
		Ventral	7	15	35	65	115
Aug. 11	Shaded	Dorsal	10	20	35	60	105
		Ventral	10	25	45	70	125
Aug. 18	Full sunlight	Dorsal	7	15	35	60	95
		Ventral	7	15	40	75	110
Aug 25	Shaded	Dorsal	7	15	35	60	95
		Ventral	7	15	30	55	85
Sept. 15	Full sunlight	Dorsal	7	15	40	75	105
		Ventral	7	15	35	60	90

TABLE 14

RELATIVE CATALASE ACTIVITY OF DORSAL AND VENTRAL BARK OF HORIZONTAL LEMON SHOOTS. SAMPLES TAKEN BETWEEN 7:30 AND 10 A.M.

Date (1924)	Tissue	Seconds required to liberate 1-5 cc. of oxygen				
		1	2	3	4	5
Shoots bent in 1923:						
July 25	Dorsal	7	15	25	45	70
	Ventral	5	10	20	35	55
Aug. 20	Dorsal	7	15	35	65	100
	Ventral	5	10	15	25	45
Shoots bent in 1922:						
Aug. 11	Dorsal	15	45	100	175	255
	Ventral	5	7	15	20	30
Aug. 15	Dorsal	10	30	65	115	170
	Ventral	5	7	10	15	20
Aug. 18	Dorsal	10	25	60	105	160
	Ventral	5	10	17	25	40
Shoots bent in 1920:						
June 23	Dorsal	7	15	30	60	105
	Ventral	5	10	15	20	30
June 25	Dorsal	12	35	85	150	240
	Ventral	5	10	15	20	30
June 26	Dorsal	10	35	70	120	160
	Ventral	5	10	15	30	45
June 28	Dorsal	15	45	105	170	250
	Ventral	5	10	15	20	30

The dorsal bark of these older shoots is much thinner, peels off less readily and has a darker color than the ventral bark.

(*c*) DISCUSSION

The results obtained with horizontal shoots justify the assumption that the initial period of sprout growth is governed by growth-promoting substances. The growing sprouts on the dorsal side seem to be able to draw on the supply of growth-promoting substances which are contained in the dorsal as well as in the ventral side of the shoot. On this basis we can account for the fact that the reflexed shoots which produced two sets of laterals, produced approximately the same amount of sprout growth as shoots which remained in the original horizontal position.

Notching above a bud on the ventral side affects only that bud, which indicates that if there is a longitudinal movement of growth-promoting substances it is slight. A notch below the bud prevents the growth-promoting substances from reaching that bud. On the dorsal side of the shoot, it is impossible to say whether or not a notch above a bud is responsible for the outgrowth of that bud, because the chances are even that it would have grown out without notching. But a notch below a bud on the dorsal side prevents growth, hence there must also be a slight longitudinal movement along the dorsal side.

The initial period of sprout growth on horizontal shoots is evidently dependent on the supply of stored food reserves. This view is strengthened by the fact that notching on the ventral side, and to some extent on the dorsal side as well, is effective only until the stored food reserves are exhausted.

With the methods employed the plant juices obtained from the dorsal and ventral sides do not show a difference in their physical properties. The data on catalase activity do not indicate that the enzyme is a factor in the initiation of sprout growth. If the small but constant differences in favor of the dorsal side of younger shoots (table 13) were significant, then we would expect sprout growth on the ventral side in older shoots because in this case the order is reversed (table 14). The reason for the great difference in catalase activity in older shoots is due to the fact that cambial activity is practically confined to the ventral side. A cross section shows this eccentric growth very clearly.

It is not clear how the initiation of sprout growth can be explained on the basis of Child's theory. We can say that by bending a shoot into a horizontal position a new metabolic gradient was set up, otherwise it would behave like a vertical shoot. But this does not furnish a basis for an explanation. Jones[12] however, obtained results with root cuttings of seakale which are in general accord with Child's conception of "metabolic gradients."

We may now consider the possibility of an inhibitory substance being responsible for the initiation of sprout growth on the dorsal side. According to this concept the inhibitory substance is produced by the growing sprouts. As in the case of vertical dormant shoots, the chief objection is that during the initial period, the sprouts are either absent or, if present, they are too small to produce enough of this substance to inhibit growth all along the ventral side. It will be recalled that on some shoots the dorsal side was kept free from sprouts for three years, yet no growth resulted on the ventral side. If we assume that there is a supply of the inhibitor in the dormant shoot which, as the shoot is bent, settles to the ventral side, it is difficult to see how this limited supply can keep the ventral side dormant for such a long time.

Furthermore, when a horizontal shoot, bearing sprouts on the dorsal side, was bent in the opposite direction, a new set of sprouts developed from the dormant buds of the previously ventral side. This new set of sprouts is unable to retard the growth of the original set throughout the initial period.

If we consider the second period of growth in contrast to the initial one, however, we find that the sprouts at the apex continue to elongate somewhat while those further back generally fail to produce the second growth cycle. We may assume that the apical sprouts produce an inhibitory substance which is responsible for this condition. But this influence is not so pronounced as in vertical shoots. When a shoot is bent, part of it remains more or less vertical. The bend does not affect the growth during the initial period, yet during the later period the rate of sprout growth at or immediately below the bend is far greater than that on the horizontal portion. As the shoot becomes older the horizontal part increases in diameter at a slower rate than the vertical part and finally ceases its growth.

VI. SUMMARY

1. The investigations here reported deal with the factors which govern the initiation of sprout growth on vertical and horizontal Eureka lemon shoots and Chinese lemon cuttings.

2. When a vertical lemon shoot is cut back to the mature wood, sprouts are produced only from the uppermost buds. The length of the sprouts decreases from the apex downward.

3. On vertical shoots, buds normally dormant can be released from dormancy by various mechanical means such as notching or girdling above a bud or wrapping tape around the upper portion of the shoot.

4. The amount of sprout growth produced is in proportion to the size of the shoot or cutting.

5. The temporary taping of the upper portion of a cutting divides it into two physiological units, each of which produces sprout growth in proportion to the size of the piece. There is no indication that the

sprouts on one portion inhibit the growth of sprouts on the other. This is also true of attached lemon shoots during the initial period of growth, but in the later growth the apical sprouts may slow up the rate of growth of subapical sprouts.

6. A dormant mature shoot or cutting contains stored food reserves in proportion to the size of the piece. With proper temperature and moisture conditions the food reserves are transformed into growth-promoting substances. This transformation is probably a gradual one which begins at the apex. When the uppermost buds are taped the supply of growth-promoting substances in that region does not become available to the growing subapical sprouts. But these sprouts are able to draw on the supply of growth-promoting substances from below.

7. This transformation of food reserves into growth-promoting substances would account for the initial period of sprout growth. The apical dominance exhibited during the later growth period may be explained on the assumption that an inhibitory substance is produced by the growing shoots which inhibits further elongation of subapical sprouts.

8. Injecting various chemical compounds into dormant Chinese lemon and Ligustrum cuttings did not affect apical dominance. Furthermore none of the substances increased the normal amount of sprout growth. Introducing cane sugar and dextrose solutions into immature Chinese lemon cuttings also gave negative results.

9. Horizontal lemon shoots or Chinese lemon cuttings produce sprouts on the dorsal side only. By bending a horizontal shoot in the opposite direction a new set of sprouts is produced from buds which would otherwise have remained dormant. By this method the total number of sprouts is practically doubled, but the total amount of growth produced is approximately the same as that produced by shoots which remained in the original horizontal position.

10. Notching above a bud on the ventral side results in sprout growth from that bud. This operation is effective only during the early part of the growing season. A notch below a bud either on the dorsal or ventral side is not effective.

11. No growth takes place on the ventral side when the buds on the dorsal side are burned out, but sprouts will appear from adventitious buds on the dorsal side. Preventing sprout growth on the dorsal side does not cause growth on the ventral side.

12. Compression or tension caused by bending a shoot has no effect upon the distribution of sprouts during the initial period.

13. No differences in the physical properties of the sap of the dorsal and ventral side were found at the beginning of the growing season. The determinations included freezing point, per cent of total solids, viscosity and hydrogen-ion concentration.

14. The relative catalase activity was slightly greater on the dorsal side of younger shoots. As the shoots become older a greater difference exists but the order is reversed.

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THE EFFECT OF A PAPER MULCH ON SOIL TEMPERATURE

BY CHARLES F. SHAW*

INTRODUCTION

Paper mulch is a term applied to a covering of specially prepared paper placed on the surface of the soil for the purpose of modifying soil temperatures, decreasing losses in soil moisture by evaporation and preventing or decreasing the growth of weeds. The paper mulch is extensively used in the Hawaiian Islands on pineapples and to some extent on sugar cane and certain vegetables. Farmers and vegetable growers in California are experimenting with the paper mulch and manufacturers are putting on the market papers specially prepared for this purpose.

There is very little literature bearing directly on this subject. The proceedings of the Annual Short Courses in Pineapple Production at the University of Hawaii[1, 2, 3] contain some discussions of the paper mulch, and a number of papers of a popular nature have been published in the news journals of the Islands. Mr. Charles F. Eckart[4] the originator and patentee of the method, and the manufacturers of mulching paper, report material benefits from its use, especially in weed control and in increased crop yields. In none of these publications is there any extended discussion of the effects on soil temperature or soil moisture. The Hawaiian Sugar Planters' Experiment Station has undertaken some studies of the effects of the paper covering in these regards, but no reports have yet been published. Unpublished data show a material increase in temperature and a considerable reduction in the loss of soil moisture where the paper mulch is used.

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* Acknowledgments are due to Professor J. W. Gilmore, who supervised the planting and supplied all crop data, and to Mr. E. V. Winterer, who cared for the thermographs and made the soil moisture determinations.

EXPERIMENTAL WORK

The experiments herein reported were undertaken to study the effects of the paper mulch on soil temperatures and soil moisture and the correlated effects on growth and development of certain crops. A plot of land about 30 × 60 ft., on the campus in Berkeley, was prepared by thorough cultivation and the removal of all stones, hard lumps and trash. Three crops were chosen for trial—milo, beans and potatoes—because they represent plants of different vegetative and fruiting habits. A further consideration was that milo is not suited to Berkeley conditions, while beans and potatoes normally do well here. The crops were each planted in three 60-foot rows placed approximately 36 inches apart. After the plants were up and well established, the paper covering was placed on the south half of the tract. The plots were prepared, the crops planted and thermographs installed early in May. The paper covering was laid on May 17, and temperatures were recorded from that date. Observations were continued to August 25 (ten days after the beans were harvested) giving a total record of 100 days.

Paper

Unperforated paper, weighing about 12 lbs. to the 100 square feet and impregnated and coated on both sides with asphaltic material, was used as the mulch cover. This was placed on the ground between the rows after the plants were well up, the paper being fitted tightly against the rows of beans and corn, and around the individual potato plants. The paper was held in place by lath placed along each edge and fastened down by long wire staples thrust into the soil. The potato plot had additional lath crossing the paper strips at intervals. It is necessary that the paper be well fastened down, otherwise it will be pulled loose and blown away by winds. Lumps and stones must be removed as they will cause the paper to break, and give access to the wind, which will tear and blow the paper. The location and layout of the plots, position of thermographs and the method of fastening the paper are shown in figures 1 to 4.

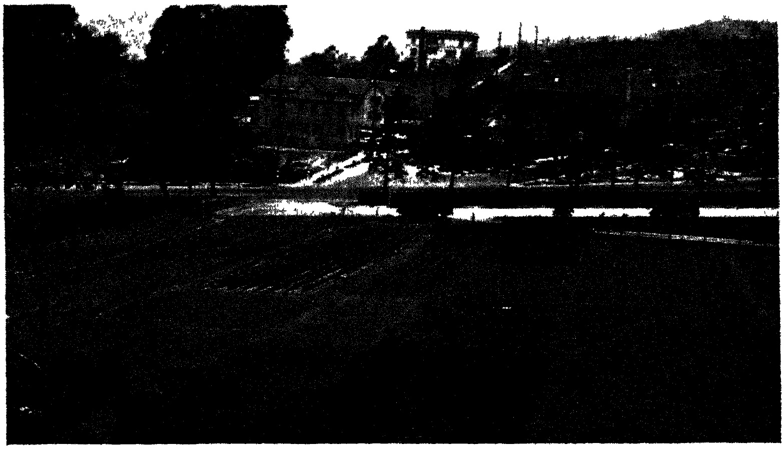

Fig. 1.—Location of covered (C) and bare (B) plots in gardens north of Hilgard Hall. The potatoes are nearest the fence (left), beans in the middle, and milo on the right.

Fig. 2.—Potato plots, June 28, 1924. Note lath on either side of the rows and across middle to hold the paper. Potato results disregarded because of uneven stand due to poor seed and variation in soil.

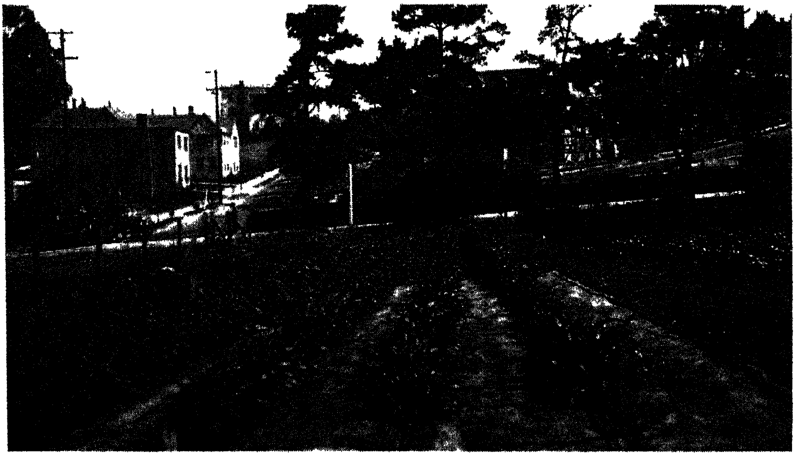

Fig. 3.—Milo plots, June 28, 1924, from the covered end. Note the lath on either side of the rows to hold the paper. These were fastened by wire staples thrust well into the ground.

Fig. 4.—Milo and bean plots, June 28, 1924, from the bare end. The two thermographs can be seen within the shelters in the bean plots.

THERMOMETERS

Two recording thermographs were used; one recording soil temperature only, the other recording both soil and air temperatures. The thermographs were placed in box shelters, mounted on posts about 18 inches above the soil surface, the thermometer bulb being buried in the soil about four feet north of the shelters, well away from any effect of shading or radiation from the shelter. The bulbs were placed in the soil in a horizontal position with the top of the bulb three inches below the soil surface, the wire tube to the thermograph being covered to a greater depth, and led up to the instrument in a wooden case. The thermographs were inspected daily, and were checked against mercury thermometers at intervals. The resulting records are complete, except for one period when the clock in one instrument was out of order; and another when the recording pen failed to leave its mark during the period of highest temperature.

WEATHER

The weather conditions throughout the experiment were normal, although the amount of fog was rather low. The Weather Bureau records show that there were 48 clear days, 17 cloudy days and 35 partly cloudy days. There was .07 in. rain on June 9, .01 in. on August 18, and a trace on August 19. During this period the winds were gentle, except on May 29 and 30, when there was a strong wind blowing from the north.

The records of the air temperatures over the plots are somewhat misleading in that the maximum on the bright sunny days is excessively high. This is due to the lack of ventilation in the instrument shelter, and the undue heating by radiation from the box covering. On cloudy days this was not noticeable, and during the night the recorded air temperatures appear to be correct.

SOIL TEMPERATURES*

The temperatures of the bare and covered plots and of the air are shown graphically in figures 5 to 11. Figure 12 shows one of the original thermograph sheets, giving the record of the covered plot and of the air for the week beginning June 30.

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* Temperatures reported throughout this paper in degrees Fahrenheit.

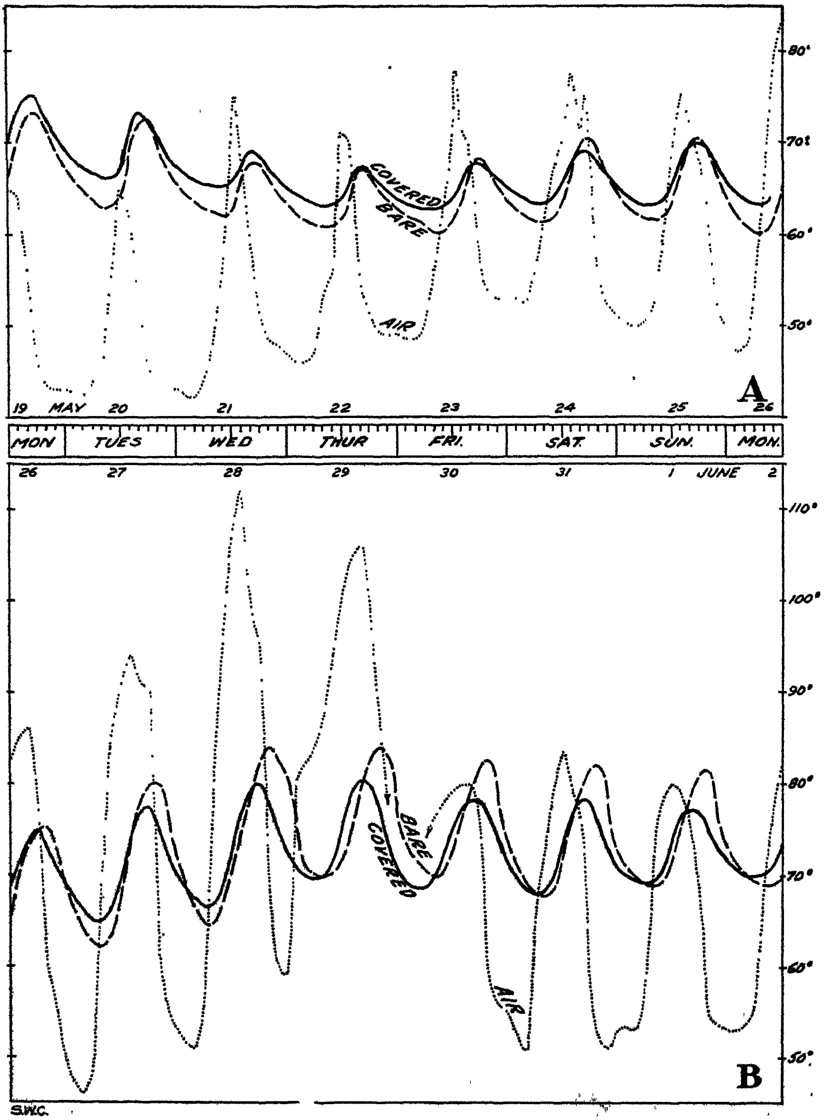

Fig. 5.—Temperature records reproduced from thermograph sheets. A—for week of May 19 to 26. B—for week of May 26 to June 2.

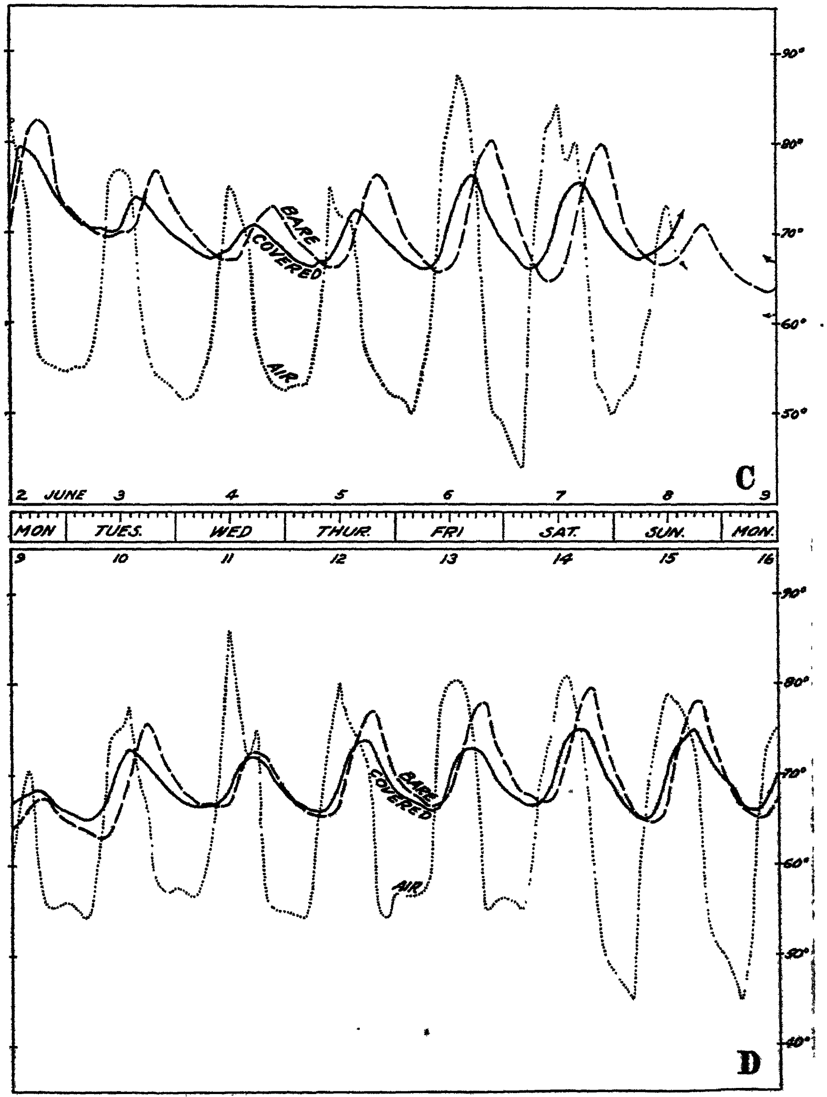

Fig. 6.—Temperature records reproduced from thermograph sheets. C—for week of June 2 to 9. D—for week of June 9 to 16.

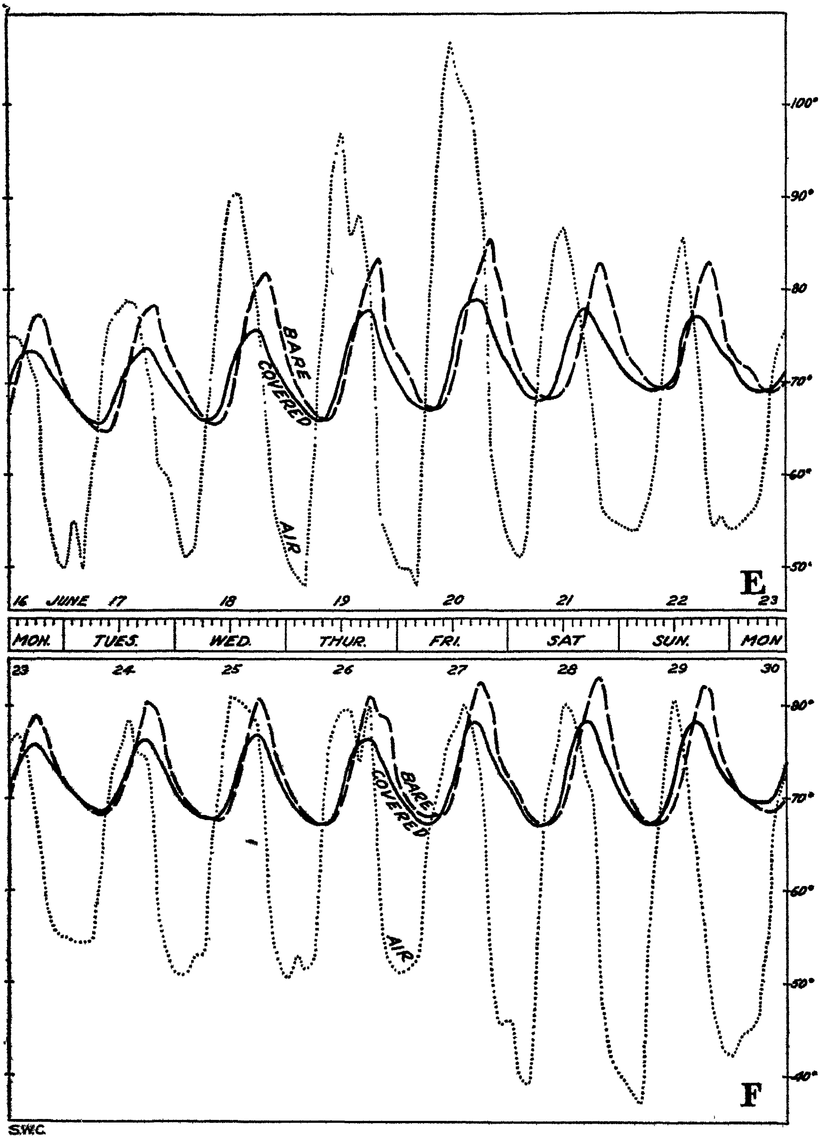

Fig. 7.—Temperature records reproduced from thermograph sheets. E—for week of June 16 to 23. F—for week of June 23 to 30.

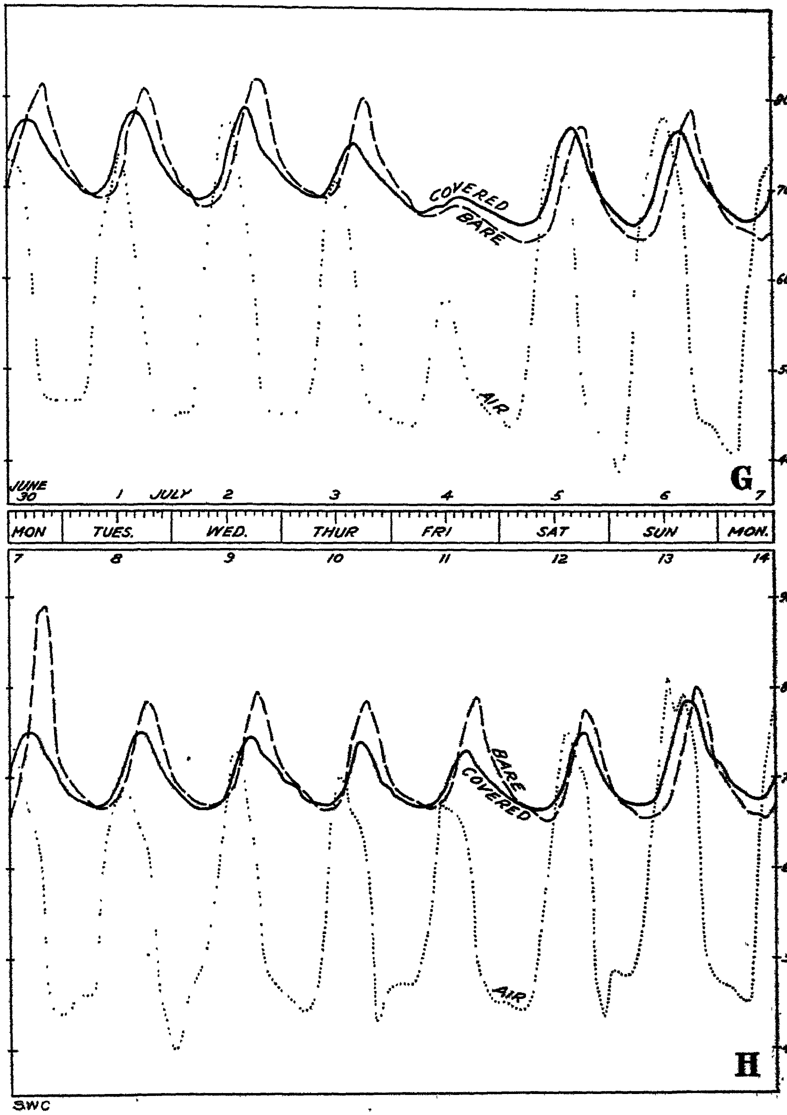

Fig. 8.—Temperature records reproduced from thermograph sheets. G—for week of June 30 to July 7. H—For week of July 7 to 14.

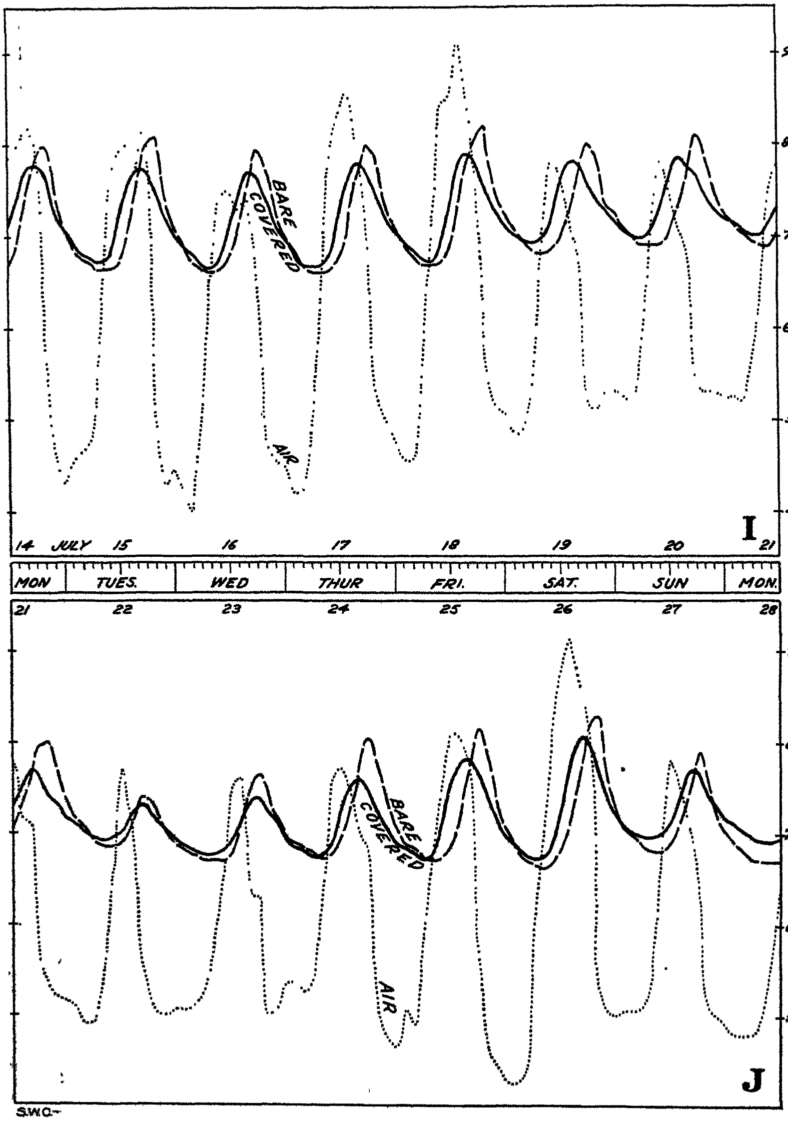

Fig. 9.—Temperature records reproduced from thermograph sheets. I—for week of July 14 to 21. J—for week of July 21 to 28.

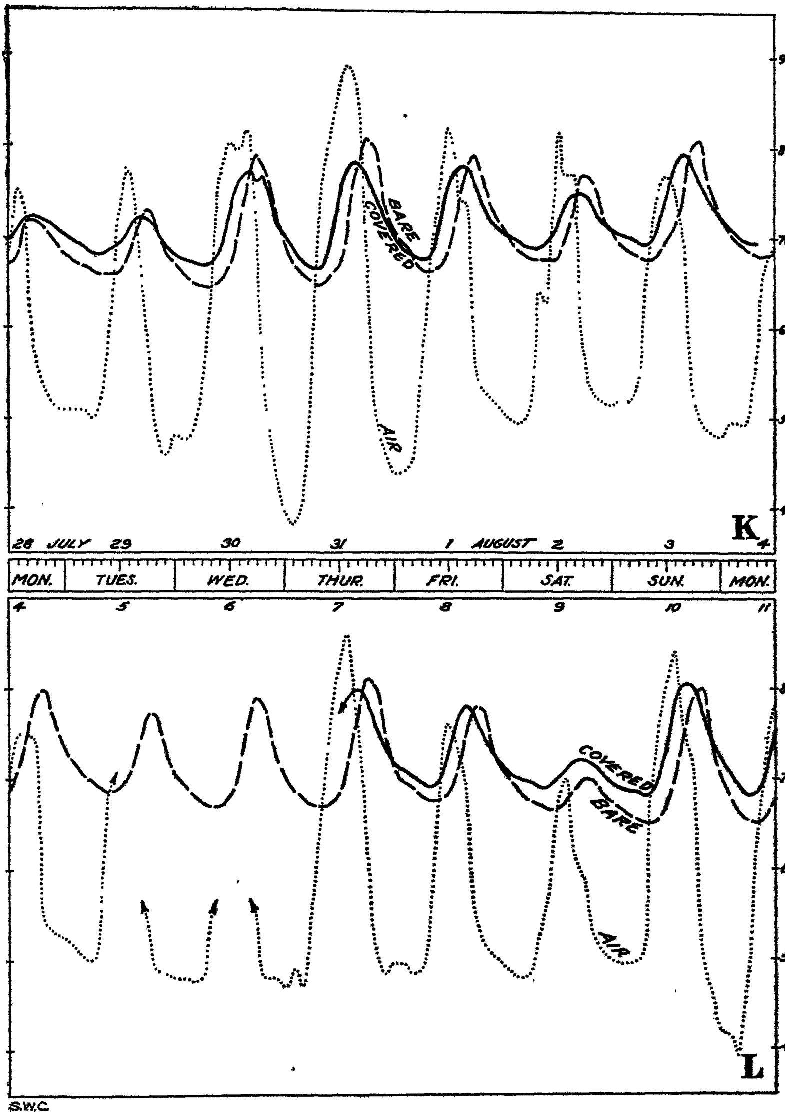

Fig. 10.—Temperature records reproduced from thermograph sheets. K—for week of July 28 to August 4. L—for week of August 4 to 11.

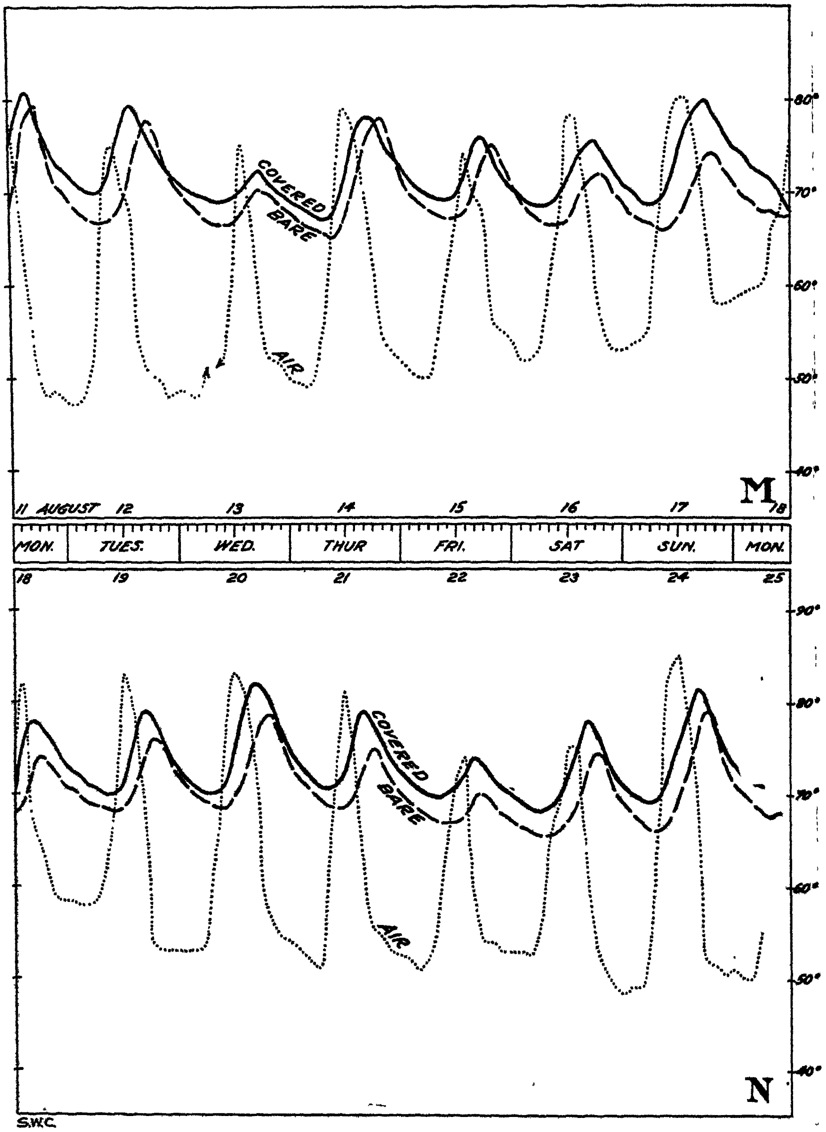

Fig. 11.—Temperature records reproduced from thermograph sheets. M—for week of August 11 to 18. N—for week of August 18 to 25.

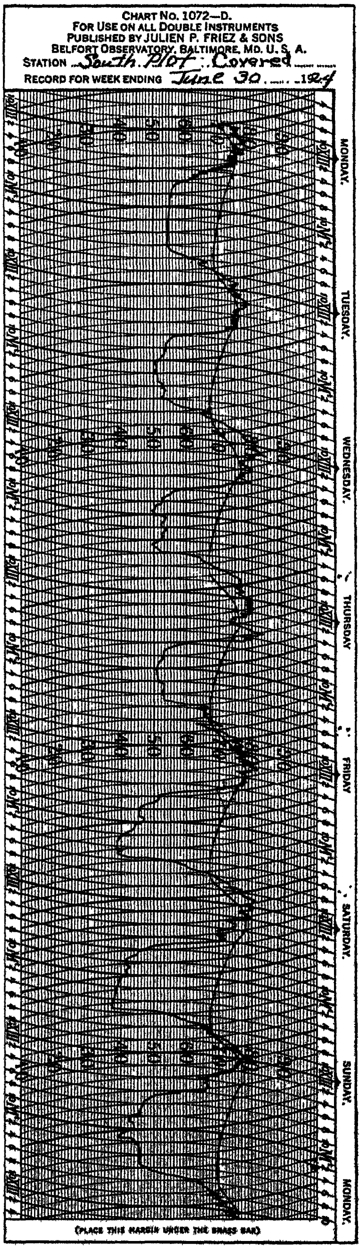

Fig. 12.—Reproduction of original of thermograph record sheet showing temperatures of the covered plot and the air for the week of June 23 to 30.

A study of these records shows the relation of the soil temperatures to that of the air. In a period of rising temperatures, with a relatively long heated period each day, as from May 25 to 29, there is a corresponding rise in both the maximum and minimum soil temperatures. The rise in the soil temperatures, although slow and of small magnitude, is definite. The effect of a single, very cold day is shown by the almost total lack of the usual afternoon rise in soil temperatures on July 4 and August 7. The soils appear to respond more strikingly to the low than to the high temperatures. This may in part be due to the more prolonged periods of low temperatures, as compared with the much briefer period of high. It may also be due in part to the damping influence of the soil depth and a lag due to heating the mass of soil above the bulbs of the thermometers. A study of the temperature records shows that there was no seasonal increase in the temperature of the soil *at this depth,* during the period of the experiment, the average weekly temperatures rising or falling slightly in response to the variations in air temperature.

The rate at which heat penetrates the soil and the effect of the covering is shown by the lag or delay of the maximum or minimum soil temperatures behind those of the air. The thermometer bulbs were covered by three inches of soil which had to be warmed by the absorbed heat before a change could occur. An analysis of the figures shows that in reaching the maximum, the covered soil had a mean lag of about 3 hours 31 minutes, while the bare plot delayed 5 hours 48 minutes after the maximum air temperature had been reached. The covered plot reached its minimum temperature 5 hours 7 minutes after the air, while the minimum of the bare plot was 6 hours 28 minutes behind the air. The total period of cooling of the covered plot, however, averaged 45 minutes longer than that of the bare plot.

The lag of the bare plot behind the covered plot is maintained quite consistently throughout the full period. Figure 13 shows the soil temperatures on two warm days—May 27 and 28—and on two cool days—July 15 and 16. On each of these four days the bare plot was two to three hours behind the covered plot in reaching the maximum temperatures, while the minimum for both plots was reached at approximately the same time each of the four days—about 8 A.M. The average hourly difference in temperature between the covered and bare plots for the full period of the experiment is shown in figure 14. The two plots averaged about the same from midnight to 2 A.M., the covered plot was .38° warmer from 2 to 4 A.M., .75° warmer from 4 to 6 A.M., 1.15° warmer from 6 to 8 A.M., 1.91° warmer from 8 to 10 A.M., 3.05° warmer from 10 to 12 noon, 3.07° warmer from 12 to 2 P.M., 1.11° warmer from 2 to 4 P.M., 1.92° cooler from 4 to 6 P.M., 3.21° cooler from 6 to 8 P.M., 1.51° cooler from 8 to 10 P.M., and .53° cooler from 10 P.M. to midnight.

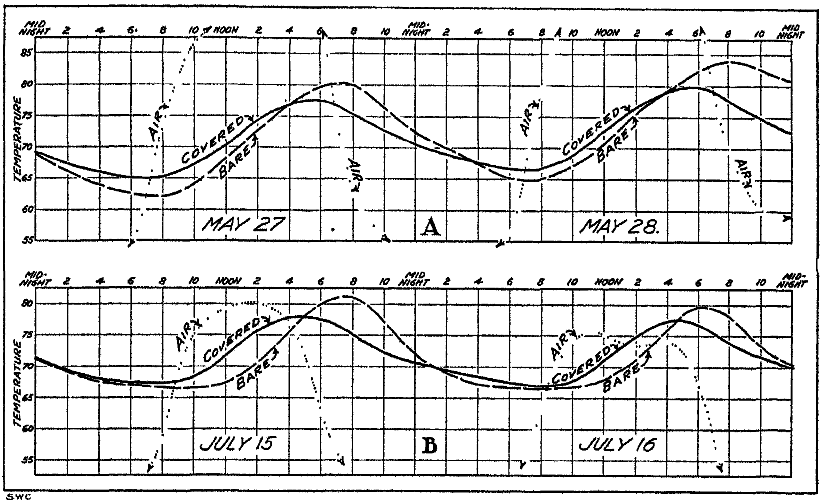

Fig. 13.—Enlarged curves showing temperatures on two warm and two cool days.

A—May 27 and 28 were two warm days, the air maxima being 94° and 112°, while the minima were 46° and 51°.

B—July 15 and 16 were two cool days, the air maxima being 80° and 75°, while the minima were 43° and 40°.

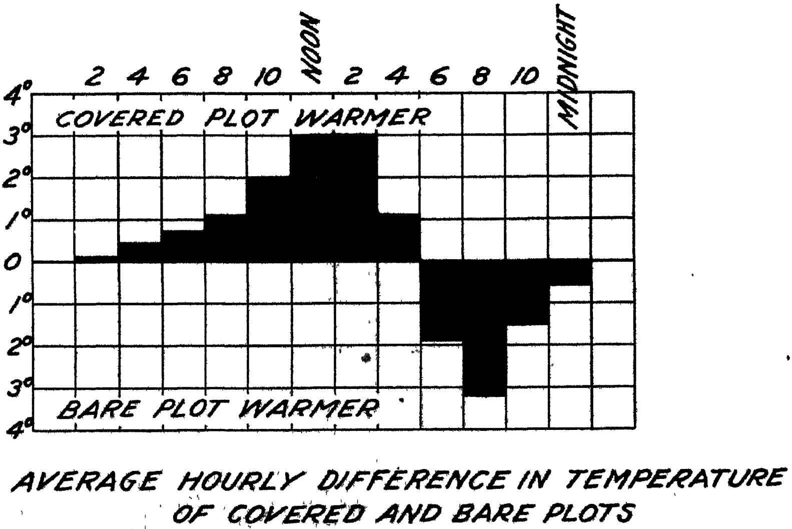

Fig. 14.—The hourly differences in temperature of the bare and covered plots, averaged for the full time of the experiment.

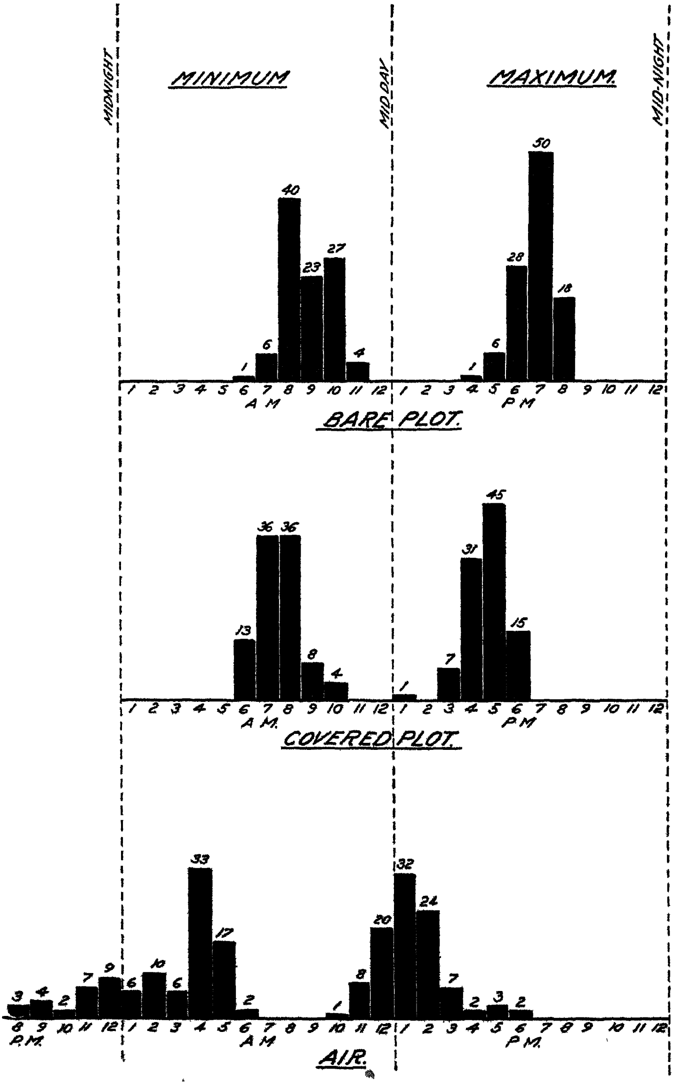

Fig. 15.—Time frequency occurrence of the maximum and minimum temperature of the bare plot, covered plot and the air.

Figure 15 shows the time-frequency occurrence of the minimum and maximum temperatures for the air and the bare and covered plots. Minimum temperatures for the air thermometer occurred irregularly between 8 P.M. and 6 A.M., the mean period being about 2:30 A.M., although the most frequent occurrences were 4 A.M. (1/3 of days) and 5 A.M. (1/6 of days). The covered plot reached its minimum about 7:30 A.M., while the bare plot was coldest about 8:50 A.M. The bare plot lagged about one hour and twenty minutes behind the covered plot in cooling to its minimum daily temperature. On three nights, however, the bare plot reached its minimum one hour earlier than the covered plot, and on sixteen nights they reached this point at the same hour.

Maximum temperatures for the air thermometer occurred irregularly between 10 A.M. and 6 P.M., the mean being about 1 P.M. and the mode lying between 12 and 2 P.M. The bare plot reached its maximum about 6:45 P.M., the mode lying between 6 and 8 P.M., while the covered plot reached its maximum temperature about 4:30 P.M., the mode lying between 4 and 6 P.M. The covered plot reached its maximum two hours before the bare plot.

The soil in both plots was warmer during the night than during the day, but the covered plot maintained a more even temperature with a much narrower range between the daily minima and maxima. The average daily range in temperature was 8.58° for the covered plot, 11.07° for the bare plot, and 31.03° for the air, while the extremes in any day ranged from 3° to 13.5° for the covered plot, from 1° to 19.5° for the bare plot, and from 14° to 61° for the air thermometer. The actual minimum and maximum temperatures reached during the period of the experiment were: Covered plot, 63° and 80°, bare plot, 60° and 84°, air thermometer, 39° and 112°. All the maxima were reached on May 29, but the minima were recorded on different dates.

NEED FOR CONTINUOUS RECORDS

In temperature studies where continuous records are not available, soil temperatures are usually read at stated intervals, often only twice or three times a day. If different plots or treatments are being compared, the results may be quite misleading. The lag of one treatment might be much greater than that of another and the time of reaching maximum or minimum temperatures might differ by an hour or more. The need for continuous records in soil temperature work is strikingly brought out by this study.

THERMAL DIFFERENCES

Thermal differences were determined by measuring on each of the original record sheets (by planimeter) the area above the 60° line as a base, and calculating the degree-hours above 60°. As neither plot cooled below 60° at any time during the experiment, there were no negative values. The results are given in table 1, which shows that the bare plot was warmer during six weeks, the covered plot warmer during eight weeks, and the two identical one week. The bare plot was consistently warmer from May 26 to July 14, while the covered plot was warmer before and after that period, the difference becoming more marked toward the latter part of the season. There is evidence that this seasonal difference may be due to variations in shading by the growing plants. The thermograph bulbs were

TABLE 1

DIFFERENCES IN TOTAL WEEKLY TEMPERATURES OF BARE AND COVERED PLOTS DETERMINED BY CALCULATING THE TOTAL NUMBER OF DEGREE-HOURS ABOVE 60° AS A BASE

Date, week ending	Hours of record	Degree-hours above 60° for the week		Degree-hours difference for week*	Degree-hours difference per hour*
		Covered	Bare		
5/19	72	588	476	+ 112	+1.55
5/26	164	1045	783	+ 262	+1.60
6/2	172	2078	2213	− 135	−0.79
6/9	169	1162	1362	− 200	−1.16
6/16	168	1497	1533	− 36	−0.21
6/23	166	1850	2032	− 182	−1.09
6/30	170	1989	2098	− 109	−0.64
7/7	168	1767	1767	0	0
7/14	167	1634	1717	− 83	−0.50
7/21	169	2031	1945	+ 86	+0.51
7/28	167	1868	1856	+ 13	+0.08
8/4	168	1868	1605	+ 263	+1.56
8/11	170	2042	1828	+ 214	+1.26
8/18	165	1945	1570	+ 375	+2.27
8/25	164	2125	1672	+ 453	+2.76
Total	2419	25,489	24,456	+1033	+ .42

**Note.*— − =Bare plot warmest; + =Covered plot warmest.

placed just west of the middle row of beans, and during mid-season were shaded to considerable extent. When the paper was put in place, the beans had developed but two pairs of leaves, and during the first three weeks there was little shading. By August first the beans were ripening, the leaves curling, and the shading was progressively decreasing. They were harvested on the 15th, and the records show that during the last two weeks—August 11–18 and 18–25, the covered plot showed by far the greatest increase of temperature over the bare plot. The totals show that the covered plot was warmer by 1033 degree-hours above 60° for the full period, or an average of .42 degree-hours per hour. A parallel study of the temperature differences (table not shown in this paper) by two-hour intervals throughout the full period, indicates that the covered plots were warmer during 1400 hours by an average of 2.16 degrees per hour, the bare plots were warmer during 634 hours by an average of 3.29 degrees per hour, while both were the same during 208 hours. The total difference in degrees, divided by the number of hours, shows the covered plot to be warmer by an average of .417 degrees per hour, closely correlating with the results obtained by the planimeter measurements given in table 1.

SOIL MOISTURE

The soil at the beginning of the experiment, on May 16, was moist and in ideal condition for crop growth. It was intended to study the effect of the paper covering on the soil moisture conditions throughout the experiment, but on May 24 the bare plot received an unauthorized irrigation, and on July 23, a broken water line flooded a portion of the covered plot. Soil moisture determinations having been started, were continued at weekly intervals throughout the full period, though it is felt that they may not be indicative of true conditions. These data are given in table 2 and indicate no moisture deficiency throughout the season, although the surface soil of the bare plots had become rather dry by August. As shown in figure 16, which gives the average moisture to a dept of 18 inches, there is a progressive decrease in the amount of water present from the beginning to the end of the experiment. The irrigation of the bare plot in the third week, and the flooding of the covered plot in the twelfth week cause characteristic breaks in the curves. From the data as presented, the paper mulch seems to have decreased the water losses from the upper eighteen inches of soil, the bare plot from the sixth to the tenth weeks containing an average of from .5 per cent to

4 per cent less moisture than that in the bare plots, or, if expressed as percentages of the total moisture present, a loss of from 4 to 20 per cent, a large part undoubtedly coming from the upper six inches. Had it been possible to sample the stony subsoil below 18 inches, this difference would be much reduced.

TABLE 2

SOIL MOISTURE IN BARE AND COVERED PLOTS

(Per cent on dry weight basis.)

Bare Plot

Depth	0–3 inches	3–6 inches	6–9 inches	9–12 inches	12–18 inches	18–24 inches
5/17	21.82%	26.46%	21.70%	20.70%	24.63%	
5/24	22.45	23.50	22.00	20.30	21.62	18.40%
5/31*	18.91	32.10	25.02	20.90	22.21	
6/7	17.53	21.02	22.99	19.44	20.00	19.91
6/14	22.75	21.22	17.91	17.05	20.00	
6/21	14.89	17.25	14.12	18.60	9.11	
6/28	14.27	18.00	16.80	21.88	10.52	5.62
7/7	13.70	15.60	14.00	15.60	11.71	17.71
7/12	6.94	13.19	12.15	13.59		22.80
7/19	12.54	14.10	12.99	10.84	12.40	17.75
7/26	12.28	15.28	14.88	13.45	13.54	16.54
8/2	6.03	13.40	13.71	12.53	17.06	19.33
8/9	8.14	10.15	12.22	12.08	14.25	13.30
8/18	5.32	8.79	14.09	11.32	12.72	16.81

* Plot irrigated by furrow method on afternoon of May 24th.

Covered Plot

Depth	0–3 inches	3–6 inches	6–9 inches	9–12 inches	12–18 inches	18–24 inches
5/17	23.10%	27.50%	24.30%	22.82%	21.72%	
5/24	14.95	23.95	20.50	19.32	18.10	
5/31	21.64	27.84	22.94	18.92	20.95	
6/7	16.25	25.13	22.05	17.83	14.39	
6/14	18.44	22.02	18.69	16.72	17.35	
6/21	15.17	19.20	17.78	15.74	20.35	20.28%
6/28	9.71	28.88	15.39	13.64	17.85	17.62
7/7	10.50	16.32	15.39	12.60	16.97	16.91
7/12	11.28	14.97	15.23	11.72	16.30	17.61
7/19	9.05	14.48	7.88	15.42	14.96	16.84
7/26	8.11	13.62	12.23	10.66	15.62	18.32
7/30*	29.85	31.65	27.00	22.39	21.72	17.51
8/2	34.10	24.25	20.90	23.78	22.49	21.10
8/9	23.40	22.22	19.35	19.70	18.64	14.78
8/18	27.10	21.23	20.15	19.86	19.15	17.95

* Covered plot partially flooded by accident on July 28, sampled on July 30. A dry, compact layer found below 15-inch depth.

The application of the water had no apparent effect on the soil temperature, there being no break or modification of the temperature curves. As the water came from pipes buried in the ground, it probably was close to the temperature of the soil at the time of application.

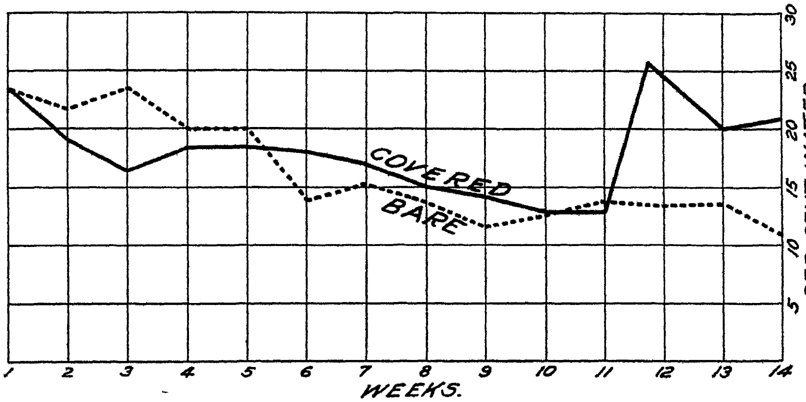

Fig. 16.—The average content of soil moisture to a depth of eighteen inches in the bare and covered plots throughout the experiment.

Crops

The crops planted were beans, milo and potatoes. Beans and potatoes do fairly well in Berkeley, and it was thought that they would give an indication of the response of a seed and a tuber crop to any differences in soil temperature which might result from the mulch. Milo was selected because it does not thrive here, it being thought that if the temperature of the covered plot was higher than that of the bare, the increased heat in this plot might result in better growth and development.

The irrigation on May 24 and the flooding of July 23 altered the crop growth to some extent, particularly that of the milo. The stand and growth of potatoes was so irregular and uneven, owing to poor seed and to soil differences that they were wholly disregarded in estimating the results. During the season the milo on the bare plot appeared a little better than that on the covered plot, but both made short irregular growths, forming heads with but few or no seeds and the evidence regarding this crop is therefore of little value.

On June 7, the beans were coming into bloom on both plots, with those on the bare plot looking somewhat the better. Five bean plants and sixteen milo plants were taken from each plot, care being taken to select uniform and average plants. The stems were cut at the ground surface and were weighed green, then dried and again weighed. The weights are given in table 3.

TABLE 3

WEIGHTS OF PLANTS HARVESTED JUNE 7

		Covered	Bare
5 bean plants	Green weight	505.6 gr.	615.9 gr.
5 bean plants	Dry weight	88.5 gr.	98.5 gr.
16 milo plants	Green weight	173.7 gr.	225.1 gr.
16 milo plants	Dry weight	26.5 gr.	32.4 gr.

On August 7, the beans were at full maturity and turning yellow, a few leaves had fallen and the pods were well ripened. Five plants were again harvested, dried and weighed with the results shown in table 4.

TABLE 4

DRY WEIGHT OF BEANS HARVESTED AUGUST 7

	Covered	Bare
Weight of beans	283.0 gr.	300.0 gr.
Weight of pods	102.0 gr.	118.0 gr.
Weight of plants	215.5 gr.	232.5 gr.
Total dry weight	600.5 gr.	650.5 gr.

The beans were harvested on August 15, and threshed on August 30. A count of 23 plants from each plot showed 506 pods from the covered and 570 from the bare plot or an average of 22 pods and 24.8 pods per plant. The total harvest is given in table 5.

TABLE 5

	Total number of plants	Yield	Equivalent yield per 100 plants
Covered	122	4.2 kg.	3.4425 kg.
Bare	208	8.8 kg.	4.2307 kg.

These various figures show that the beans did considerably better on the bare plot, with the milo giving indications in the same direction. The accidental irrigation may have modified these results somewhat, but as neither plot showed any deficiency of water during the season, the results of growth and yield are considered representative of the effects of the treatment.

The fact that the paper was not perforated, and aeration therefore restricted to some extent, might account for some differences in yield and growth. The papers used in mulching are usually perforated.

CONCLUSIONS

Covering the soil with an asphalt-coated paper increased the mean temperature of the soil by an average of about .42 degree per hour. The covered plots were warmer 62.5 per cent of the time, the bare plots warmer 28.3 per cent of the time, and they were the same about 9 per cent of the time. The covering hastened the time of warming, retarded the rate of cooling, and gave a narrower range between the maximum and minimum temperatures with a resulting more uniform temperature condition. The experiment demonstrates that a paper covering modified the delay or lag in reaching maximum or minimum soil temperature and emphasizes the need for continuous records in any soil temperature studies where differences in treatment or shading may occur.

Soil moisture losses from the upper eighteen inches that were sampled were reduced to an appreciable extent by the paper covering, much of the loss from the bare plot apparently being due to the drying out of the upper six inches. The water present at the end of the experiment was still above the wilting point and there was no moisture deficiency in either plot. Crop yields indicate that the covering is of no benefit to any of the crops grown, the figures actually indicating an adverse effect.

From the results of this experiment, it is evident that while the use of the paper mulch cover may conserve the moisture to some extent, they give no indication that it will favorably affect the growth of crops under such climatic conditions as exist in Berkeley.

SUMMARY

The paper mulch is extensively used in the Hawaiian Islands, and is being tried out in other parts of the United States. An experiment was carried out in Berkeley, using a paper of medium weight as a mulch, with potatoes, milo and beans as crops.

Thermograph bulbs were placed in the soil at the dept of 3 inches below the surface, and continuous records of soil temperatures for the covered and bare plots obtained.

The temperatures show that the *covered* plot lagged about one and one-third hours behind the bare plot in reaching the minimum temperature, while the *bare* plot lagged about two hours behind the covered plot in reaching the maximum.

The average daily range for the covered plot was 8.58°, and for the bare plot was 11.07°. The covered plot averaged about .42 degree per hour warmer than the bare plot. The use of paper gave more uniform and slightly higher soil temperatures.

The soil moisture gradually decreased during the season, except when accidental irrigation increased the supply. The covered plots lost water more slowly than the bare plots but neither showed any deficiency of moisture during the period of the experiment.

The growths of potatoes and of milo were unsatisfactory and the yields were not considered. The beans did considerably better on the bare plot.

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POLYEMBRYONY, HETEROZYGOSIS AND CHIMERAS IN CITRUS[1]

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INTRODUCTION

The genus Citrus is characterized by remarkable genetic variability, both in seed reproduction and within clonal varieties. An F_1 hybrid progeny usually exhibits great genetic diversity (Swingle, 1913*a*), suggesting the F_2 generation from a cross between races differing in many genes. Bud-variation types arise frequently, and involve changes in many characters of tree and fruit (Shamel *et al.,* 1918, 1918*b*, 1920).

With any tree fruit, considerations of time and expense seem to make thorough genetic analysis impracticable; with Citrus, polyembryony appears to render it even theoretically impossible. Such remarkable genetic phenomena, however, occurring in a group of such great economic importance, deserve the best interpretation possible on the basis of the theory of heredity worked out with organisms more suitable for genetic study.

The present paper reports evidence, obtained from the pedigree cultures of Citrus at the Citrus Experiment Station, bearing on the relation of apogamy to genetic variation in Citrus. It also includes preliminary data which can best be presented in a general publication preceding detailed reports on limited problems. A comprehensive review of the literature of Citrus genetics is not attempted, but the principal published evidence is briefly discussed, and an attempt is made to evaluate its general significance and to indicate clearly the outstanding problems.

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[1] Paper No. 135, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

The various Citrus forms mentioned in this paper will usually be designated by their ordinary English names. These names, with the corresponding Latin names according to Swingle (1914, 1914*a*, 1915) except for the species recently discussed by Merrill and Lee (1924), are as follows: kumquat, *Fortunella* spp.; lemon, *C. Limonia* Osbeck; mandarin, tangerine, etc., *C. nobilis* and varieties (King mandarin or orange, *C. nobilis* Lour.; Willow-Leaf or China mandarin and Dancy tangerine, *C. nobilis* var. *deliciosa* Swingle; satsuma, *C. nobilis* var. *Unshiu* Swingle); sweet orange (often called simply "orange" in this paper), *C. sinensis* Osbeck; sour orange, *C. Aurantium* L.; pummelo (including grapefruit and shaddock), *C. maxima* (Burm.) Merrill. The citranges are hybrids between *C. sinensis* and trifoliate orange (*Poncirus* (*Citrus*) *trifoliata* Raf.).

Since the "Satsuma orange," or "Satsuma mandarin," is classed by Swingle as a distinct botanical variety, and includes several horticultural varieties (Scott, 1919), the name *satsuma* is here used as a common noun, synonymous with the Japanese *unshiu*. There seems to be no good English group name for the King type.

In accordance with Swingle (1914*a*, 1916) and Merrill and Lee (1924), *grapefruit* is used for the type of *C. maxima* (var. *uvacarpa* Merrill and Lee) commonly cultivated in America. The general American public has not accepted *pomelo* as the name of this fruit, and the authors just cited show that its acceptance is not desirable, since *pummelo* (Swingle, 1916), spelled in various ways, is widely used in other countries for the shaddocks and forms intermediate between shaddocks and grapefruit. Since it is very convenient to have one English name that is applicable to the whole species, and *pummelo* is in use for a great range of types, while *grapefruit* and *shaddock* are definitely established in the United States and the West Indies to designate extreme forms, it seems desirable to include under the term *pummelo* all forms belonging to the species *Citrus maxima* (*C. grandis* Osbeck). Swingle (1916, p. 2751), in one brief reference, seems to advocate this delimitation of the term, although elsewhere he (1914*a*, 1916) excludes the grapefruit, which is little grown in the Oriental regions where the name *pummelo* is in general use. The somewhat extensive use of *pomelo* for the grapefruit in American horticultural literature is a further reason for applying *pummelo* to the whole species, instead of excluding the grapefruit. This application of the term is of decided advantage in horticultural naming of hybrids of grapefruit; *tangelo* (from *tangerine* and *pomelo*) is well established in horticultural literature, and such forms as *lemelo* and *mandelo* are likely to be needed.

In the following discussion of Citrus genetics, *it is concluded, from the available evidence, that Citrus forms are in general extremely heterozygous, as the Hagedoorns (1914) have suggested. This conception seems highly significant in relation to various aspects of Citrus genetics. In the evolution of this heterozygosis, polyembryony probably was an important factor.*

I do not wish to appear, however, to prejudge the case, either in general or with reference to any particular instance or type of variation here considered. Certain alternative explanations and possible objections will therefore be mentioned at this point.

Some of the variations are certainly very remarkable; for example, Swingle's (1913*a*) lemon-*trifoliata* hybrids which bear hypophylls instead of normal leaves, and his citranges bearing many leaves with five leaflets. The different combinations in F_1 hybrids from one cross (as the citranges of Webber and Swingle), of various parental characters, even of ones that seem to be respectively characteristic of the parent species, suggest the variability of dominance which Swingle has inferred to exist. It may be that trees with extra chromosomes occur frequently among the sexual progeny[2] generally, as it appears that tetraploid trees do among the apogamic progeny in our cultures. This or some other exceptional or little known cause may account for some of the striking variations among hybrids. "Zygotaxis," the hypothesis proposed by Swingle (1913*a*) as a possible general explanation of the great variability of Citrus hybrids, is especially discussed below, in the section on "Heterozygosis."

Recent work on Oenothera cytology (S. H. Emerson, 1924) indicates that chromosome behavior in that genus differs widely from the Drosophila type which seems so widely prevalent. In a few of our parent varieties which have been examined cytologically, however, it appears that the chromosomes usually pair and separate normally at meiosis in the pollen mother cells, and as a rule produce normal-appearing pollen tetrads. It is therefore improbable that a majority of their hybrids have aberrant chromosome numbers, unless chromosome elimination after fertilization is very common in hybrids. The high variability of F_1 Citrus hybrids is, however, a very general phenomenon, not limited to a minority of the individuals. Further, enough is known, in general, of the production of new characters by new combinations of genes in crossing, to warn us against setting any narrow limits to the probable results of recombination in crosses between two highly heterozygous species. On the other hand, a new warning against undue confidence in gene stability is given by Eyster's (1924) recent hypothesis of qualitative division of certain genes. Possibly genes specially affecting other characters are sometimes unstable in the same way as are certain genes relating to variegation. If Citrus is especially notable for the occurrence of such unstable genes, this fact may account for part of the remarkable variability that is observed.

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[2] In the interest of conciseness, the terms *apogamic* and *sexual* will frequently be used in this paper, combined with such words as *embryo, seedling,* and *progeny,* in the sense of "produced by apogamy" and "produced by fertilization," respectively.

POLYEMBRYONY

Citrus seeds are frequently polyembryonic. Strasburger (1878, 1907) showed that the supernumerary embryos are formed by proliferation of nucellar cells surrounding the embryo sac. These adventitious embryos may be expected, therefore, to reproduce the seed-parent genotype, without variation due to segregation in sporogenesis or to recombination in fertilization.

Citrus polyembryony is not entirely due to nucellar budding, however, for in 10 (probably 11) cases in our cultures, among more than 1000 hybrids, two hybrid seedlings have come from one seed. The seeds were planted separately, and all operations on which the reliability of the pedigrees depended were so carefully performed and checked that the single-seed origin of the pairs of seedlings is beyond doubt. The budding and consequent labeling were done with similar care. In eight of the ten cases, both of the original seedlings, as well as trees budded from them, have been positively classified as hybrids; in the ninth case, both of the small seedling trees are almost surely hybrids, as the budded trees certainly are; and in the tenth case one seedling died undescribed, so that its record depends on budded trees alone. In the eleventh case, one of the two "seedlings" died young, and their separateness below the surface of the soil was not proved. Since in every case the two hybrids seem to be identical in type, in spite of the usual great diversity among hybrids of the same parentage, it is probable that these are all cases of "identical twins," each pair being derived from one fertilized egg. The seed-parent varieties that have produced identical-twin hybrids are: King, Owari satsuma and Willow-Leaf (*C. nobilis*); Ruby and Valencia (*C. sinensis*); and Imperial (*C. maxima*). Part of the pollen parents are indicated in footnote *a* to table 2; those concerned in the three cases from earlier cultures were Dancy and Willow-Leaf.

Less than one per cent of our hybrid-producing seeds have given two hybrids each. Since the apogamic progeny from crossing (recognized by their strictly maternal characters) have been nearly three times as numerous as the hybrids, it is plain that the fission or budding of sexually produced embryos plays only a minor part in the total production of supernumerary embryos. Since the number of apogamic embryos per seed is indefinite, occasional fission in apogamic embryos may occur, but could be detected only by microscopic examination.

As is shown by the data of table 1, Citrus seeds are highly variable in number of embryos. The embryos examined were highly variable in size and often irregular in shape. Some were very small, and possibly some smaller ones escaped observation.

There must be, therefore, much opportunity for competition among embryos within Citrus seeds, and it may be that many are eliminated at early stages of development. There is plainly much opportunity for the fertilized egg to be crowded out by apogamic embryos. The chances of such elimination must depend largely on the number of adventitious embryos that start, and on the position and the relative age and vigor of the two classes of embryos. Comparison of table 1 with the *Total seedlings* column of table 2 indicates that very many of the apogamic embryos fail to germinate. Germination must therefore give much opportunity for selective elimination; survival may be determined by differences in size, vigor, position, morphological completeness, and susceptibility to infection. Albinism (pp. 377–379) causes the early death of many of the seedlings from some parents.

Citrus presents therefore one form of the "developmental selection" (natural selection acting within the soma of the parent) whose evolutionary significance has been discussed by Buchholz (1922). Many of the embryos from fertilized eggs must compete with apogamic embryos in the same seed, and with such embryos in other seeds of the same fruit. Genotypes inferior in vigor to the seed parent must be more severely handicapped in Citrus than in forms where competition is between ordinary monoembryonic seeds. On the other hand, viable sexual embryos are entirely unnecessary for reproduction, provided apogamic embryos are able to develop. The added difficulty

TABLE 1

NUMBER OF EMBRYOS PER SEED IN TEN SEEDS OF EACH OF TEN CITRUS VARIETIES

	Frequency (a)												Mean embryos per seed
Embryos:	1	2	3	4	5	6	7	8	9	10	11	12	
King mandarin(*b*)	10												1.0
Sweet lemon(*b*)	9(*e*)	1(*d*)											1.1
Lisbon lemon(*b*)	8(*e*)	2(*e,f*)											1.2
Eureka lemon(*b*)	8	1(*d,e*)	1(*d*)										1.3
Ruby (blood) orange(*c*)	3(*e*)	5	1	1									2.0
Mediterranean Sweet orange(*c*)	3	4(*d*)	2				1						2.4
Dancy tangerine(*c*)	3	1	4		1	1							2.8
Imperial grapefruit(*c*)	1	1	2(*e*)	3(*e*)	1		1	1					4.1
Same, fruit No. 6(2) (*c,g*)	4(*d*)	3	3										1.9
Owari satsuma(*c*)			1		6(*d*)			2	1(*d*)				5 8
Willow-Leaf mandarin(*c*)			2		2	2	1	1(*d*)		1(*d*)		1	6 5

(*a*) A few doubtful decisions are included without special indication. Except as noted, the embryos had two cotyledons each.

(*b*) Random samples, each from mixed seed of ten fruits of the crop of 1925, from open pollination; dissection and description by A. C. Austin.

(*c*) Random samples, each (with the one listed exception) from mixed seed of several fruits of the crop of 1917; dissection and description by M. H. Roblee. Satsuma seeds from open pollination, the rest from selfed (bagged) flowers.

(*d*) In each case one embryo with only one cotyledon.

(*e*) In each case, one embryo with three cotyledons.

(*f*) One embryo with four cotyledons.

(*g*) A fruit with remarkably short, small seeds.

in Citrus breeding which results from the occurrence of apogamic embryos has been pointed out by Webber (1900).

It seems, however, that apogamic embryos do not often develop in the absence of fertilization. Strasburger (1907) states that fertilization precedes the formation of adventitious embryos, and that, while the latter are usually present, the sexually produced embryo is seldom absent. Webber (1905) reports that seeds have occasionally resulted from flowers protected from pollination, but considers that fertilization is usually a prerequisite for seed formation. In ordinary solid plantings of the Washington navel orange, a variety which, according to Osawa (1912), produces a few good embryo sacs but no pollen at all, seeds are very rare; yet, in my work and elsewhere (Coit, 1915), fruits from artificially pollinated flowers of this variety have very often contained seeds. I have obtained similar results with a variety of satsuma (evidently Owari; Scott, 1919) which from its usual seedlessness, the appearance of its pollen, and Osawa's (1912) cytological study of the "unshiu" appears to have little or no functional pollen. Out of 79 satsuma fruits from artificial cross-pollination by "seedy" varieties, 66 contained seeds, while 34 fruits from flowers bagged for selfing on the same trees in the same two seasons were all entirely seedless. The fruits produced by varieties with good pollen, similarly bagged for selfing, usually contained seeds. Other observations agree with these.

Altogether, it seems very probable that Citrus seeds do not often develop without pollination, although seedless fruits sometimes develop without pollination even in varieties normally seedy. In view of the abundant production of adventitious embryos, this fact is somewhat surprising.✓It would appear (Strasburger, 1907; Webber, 1905) that the nucellar budding which produces the adventitious embryos is at least very largely dependent on some growth stimulus due to the fertilized egg. Although certain species hybrids, such as many citranges (Swingle, 1910) and the Sampson tangelo, seem to give apogamic progeny exclusively when selfed, this fact does not demonstrate their ability to form apogamic embryos without fertilization.

In crosses between species, where the hybrids can usually be positively distinguished from the apogamic progeny, we may expect (see p. 369) to find a negative correlation between the percentage of hybrids and the amount of apogamy characteristic of the seed-parent species—and also between percentage of hybrids and characteristic vigor of apogamic seedlings.

TABLE 2

NUMBERS OF TOTAL SEEDLINGS PER SEED AND OF HYBRID SEEDLINGS PER SEED, FROM INTERSPECIFIC CROSSES

Crosses involving the same seed parent combined. (*a*)

Seed-parent variety(*b*)	Number of pollen-parent varieties(*b*)	Number of seeds giving seedlings	Total seedlings (per cent of seeds)	Hybrid seedlings (per cent of seeds) (*c*)
Sweet lemon	2	22	100	100
Lisbon lemon	2	62	106	83.9±3.1
King mandarin	5	332	100.6	79.8±1.5
Eureka lemon	2	119	108	73.9±2.7
Mediterranean Sweet orange	3	105	110	61.0±3.2
Ruby orange	1	42	119	47.6±5.2
Imperial grapefruit	5	503	128	46.7±1.5
Valencia orange	2	57	135	28.1±4.0
Owari satsuma	3	193	139	21.2±2.0
Willow-Leaf mandarin	5	714	127	18.6±1.0
Dancy tangerine	2	54	126	18.5±3.6
Navel oranges (2)	2	55	136	7.3±2.4

(*a*) This table includes only classified progeny; the actual germination, and probably the number of seedlings per seed, were greater, since a large number of young seedlings died from various causes, including albinism. In seven certain cases and one probable case of the production of two hybrids from one seed, the seeds concerned are omitted from the tabulation. There were two cases in series 120 (see table 3 for parentage of series), and one each in series 24, 72, 100, 104 (the separateness of the two "plants" was not proved), 107 and 119.

(*b*) Including all in the cultures of 1917, excepting the cases of intraspecific crosses.

(*c*) The probable error is obtained from $.67449\sqrt{\frac{pq}{n}}$, where p is the observed percentage of hybrids, q is $1-p$, and n is the corresponding total number of seeds giving classified seedlings.

Table 2 gives data bearing mainly on the former point, for all the available series of the hybrid cultures of 1917,[3] arranged in the order of the percentage of hybrids. When we consider both the data of table 1 and the actual "percentage of seedlings," it is evident that viable hybrids tend to become scarcer as embryos become more numerous. The two varieties most conspicuously polyembryonic in table 1 are among the lowest in number of hybrids in table 2. On the other hand, the five varieties that are lowest in embryos are highest in hybrids. The differences in hybrids between these two groups of seed parents are, in general, highly significant statistically. The former group produced a high percentage of seedlings, and the latter group a low percentage, thus confirming the indications of table 1 as to characteristic numbers of embryos.

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[3] All lots are included except those from intraspecific crosses. There are a few cases of doubtful classification, but it is very improbable that the final results will make much change. The classification has been made by tree characters throughout, but it has already been confirmed in very many cases, and rarely corrected, on examination of fruit.

With the intermediate varieties of table 1, the correlation is much less regular, but the hybrid percentages are in most cases intermediate between those of the groups just discussed. Dancy tangerine (embryos medium) and the navel oranges (no embryo count) have both given a high seedling percentage (see also Coit, 1915, p. 58) and a low hybrid percentage; in both cases the young apogamic seedlings are decidedly vigorous, and competition may be especially severe in proportion to the number of apogamic embryos present. The most marked exception to the general trend of the results is Imperial grapefruit, which has a rather high hybrid percentage in spite of its rather high embryo count, its high seedling percentage, and the decided vigor of its apogamic seedlings; this variety is, however, definitely intermediate between the two groups first mentioned in both embryos and hybrids.

Mediterranean Sweet seems (table 1) to produce a considerable number of apogamic embryos when selfed, yet it has given a low seedling percentage and a high hybrid percentage. This may be a result of its relatively low vigor of growth, as shown both by orchard trees and by young apogamic seedlings. All the other orange varieties of table 2 have produced more numerous and more vigorous apogamic seedlings, and a smaller percentage of hybrids.

The promptness with which apogamic development begins after fertilization may differ in different varieties, and differences in this respect may affect the proportion of viable fertilized eggs.

The negative correlation between total seedlings and hybrid seedlings shown by the tables may be due in part, of course, to more extensive elimination of *apogamic* embryos in lots where the sexual embryos are especially vigorous in comparison. Thus the recorded medium embryo count for Mediterranean Sweet may be fairly representative for selfing, but not for crossing. It should be noted, however, that in the orchard the hybrid progeny of this seed parent are frequently somewhat *less* vigorous than the apogamic progeny.

There remains the general question of how far the proportion of hybrids may be affected by the pollen parents. Table 3, which segregates the data of table 2 by pollen parents, suggests an approach to random-sampling variation among the lots from each seed parent,

TABLE 3

NUMBER OF TOTAL SEEDLINGS PER SEED AND OF HYBRID SEEDLINGS PER SEED

Data of table 2, progeny of each cross given separately.(*a*)

Seed-parent variety (clone)	Series	Pollen-parent variety (clone)	Number of seeds giving seedlings	Total seedlings (per cent of seeds)	Hybrid seedlings (per cent of seeds) (*b*)
Lisbon lemon	91	Valencia orange	25	100	88.0±4.4
	92	Imperial grapefruit	37	111	81.1±4.3
King mandarin	97	Genoa lemon	18	106	72.2±7.1
	98	Lisbon lemon	4	(100)	(100)
	99	Mediterranean Sweet orange	166	100	85.5±1.8
	6	Valencia orange	35	103	60.0±5.6
	100	Imperial grapefruit	109	100	78.0±2.7
Eureka lemon	89	Valencia orange	37	114	62.2±5.4
	90	Imperial grapefruit	82	105	79.3±3.0
Mediterranean Sweet orange	113	King mandarin	36	111	63.9±5.4
	114	Willow-Leaf mandarin	50	112	54.0±4.8
	116	Imperial grapefruit	19	105	73.7±6.8
Ruby orange	72	Dancy tangerine	42	119	47.6±5.2
Imperial grapefruit	117	"Hedge bergamot" (*c*)	108	111	54.6±3.2
	118	Eureka lemon	6	(117)	(17)
	119	Lisbon lemon	73	121	63.0±3.8
	120	Willow-Leaf mandarin	270	136	41.9±2.0
	121	Orange (blood, tree N102)	46	137	34.8±4.7
Valencia orange	22	Dancy tangerine	21	138	9.5±4.3
	24	Willow-Leaf mandarin	36	133	38.9±5.5
Owari satsuma	101	Lisbon lemon	2	(150)	(50)
	54	Valencia orange	82	139	20.7±3.0
	102	Imperial grapefruit	109	139	21.1±2.6
Willow-Leaf mandarin	103	Lisbon lemon	6	(117)	(17)
	104	Ruby orange	172	124	21.5±2.1
	105	Valencia orange	192	128	12.0±1.6
	106	Orange (blood, tree N102)	59	131	30.5±4.0
	107	Imperial grapefruit	285	128	18.9±1.6
Dancy tangerine	95	"Hedge bergamot" (*c*)	7	(114)	(0)
	96	Imperial grapefruit	47	128	21.3±4.0
Washington orange	108	Willow-Leaf mandarin	17	141	5.9±3.9
Orange (navel, tree N1).	110	Dancy tangerine	6	(133)	(17)
	111	Willow-Leaf mandarin	32	134	6.25±2.9

(*a*) The 22 progeny of Sweet lemon, omitted here, were from pollination by Mediterranean Sweet orange (7 trees) and Imperial grapefruit.

(*b*) The probable error is obtained as in table 2.

(*c*) A peculiar form with brachytic shoots, occasionally used for hedges in California. It is very unlike typical *Citrus bergamia* Risso, and may be closer to sour orange; it resembles the form which Risso and Poiteau (1818–22) described under the name *C. bigaradia crispifolia.*

although some of the differences appear statistically significant. This table shows clearly that the more significant differences of table 2 are not due to differences in fertility or viability with different pollen parents. This fact is especially well shown by comparison of Imperial with all other pollen parents (table 3). With four seed parents, Imperial alone (table 3) has given hybrid percentages very similar to those given by the combined pollen parents (table 2); and with Eureka and Mediterranean Sweet the differences between pollen parents are less than three times their probable error. On the other hand, the cross Imperial by Willow-Leaf has given more than twice the hybrid percentage of the reciprocal cross, and the difference is about nine times its probable error.

Even in the case of differences between pollen parents that appear statistically significant (none are unequivocally so), the indications as to differential fertility or viability are very dubious, because of the probability of non-random differences between fruits in the percentage of hybrids. That is, the variability of the percentage of hybrids from any cross, among the lots of seedlings produced by the respective seed-parent fruits, may tend to be greater than is to be expected from the general percentage of hybrids among the total progeny from the cross in question. Such a situation may exist if the physiological conditions favoring apogamy, within a given seed-parent variety, vary markedly by whole fruits or branches, since in this case the variability, in number of embryos, *of the seeds taken by single-fruit lots,* will tend to be greater (Fisher, chap. 10) than if the fruits were substantially random samples of seeds from one statistical population for amount of apogamy. Such high variability among fruits in amount of embryonic competition would be expected to give high variability in the percentage of viable hybrids. In this case, wide differences in hybrid percentage, in different crosses involving the same seed parent, would be less significant than if the fruit lots of seeds were random samples with respect to viable hybrid embryos.

Statistical study of the variability of the percentage of hybrids is needed, but must be deferred until the records of the numbers of hybrids have been completely checked and revised on the basis of fruit characters. Some of the records are so suggestive, however, as to justify mention of the hypothesis just stated. An especially striking illustration may be added. With Willow-Leaf as seed parent, the hybrids are often so grouped with reference to the parent fruits as to suggest that the variation in embryonic competition among the fruits was by no means random. For instance, series 104 gave a total of 37 hybrids from 172 seeds, but three relatively few-seeded fruits from one bagged branch gave 10 hybrids from 13 seeds, and one other fruit gave (excluding the case of identical-twin hybrids) 6 hybrids from 8 seeds. Thus the other 151 seeds reported in table 3 gave only 21 hybrids. The fruits mentioned gave a total-seedling percentage of only 100, while the other 151 seeds gave a percentage of 128. Evidently those four fruits had seeds with relatively few apogamic embryos, in which the hybrids encountered little competition. Table 1 mentions an Imperial fruit whose seeds were small and had comparatively few embryos.

With each of the first four seed parents of table 3, the crosses higher in total seedlings are generally lower in hybrids. The numbers are evidently too small, however, to make these differences significant even when taken together. Imperial shows similar differences, except that the two lots relatively low in seedlings show the reverse difference in hybrids between themselves. Satsuma shows practically no difference between pollen parents. The differences in hybrids with Willow-Leaf, with relatively large numbers, show no definite relation to the differences in total seedlings.

It is probably significant in this connection, that King and the lemons, which have few extra embryos, have rather frequently given weak hybrids when used as seed parents in interspecific crosses, while with satsuma and Willow-Leaf, which have many embryos, weak hybrids seem decidedly less common. In the latter case, presumably, the severity of the apogamic competition seldom permits weak hybrid embryos to survive.

It may fairly be concluded that the differences in the percentage of hybrids depend mainly on the seed parents.

We therefore have experimental evidence indicating that Citrus varieties differ greatly in abundance of apogamic embryos, and that, in varieties which produce relatively numerous apogamic embryos, the embryo resulting from fertilization is relatively often crowded out. There is also some indication of a negative correlation between vigor of apogamic embryos and percentage of viable sexual progeny. These considerations have an important bearing on breeding procedure. Varieties which produce relatively few adventitious embryos can be more economically used as seed parents. Counts of embryos often give valuable indications in this connection.

VARIATION AMONG SEEDLINGS PRODUCED BY APOGAMY

In our pedigreed cultures, albino and partially albinistic seedlings have occurred in various lots of progeny, both from selfing and from crossing, and sometimes in surprising abundance. In some cases (fig. 1) two such seedlings came from the same seed, so it is very probable that at least one of these, in each case, was produced by apogamy. In many cases the same seed produced both albinistic and green seedlings. Some seedlings are light green; others are partly green and partly white, but these often seem to lack the definite delimitation of ordinary variegation. Apparently no seedlings long survive unless they produce fully green leaves at a very early stage. No cases of true variegation have been observed among older seedlings.

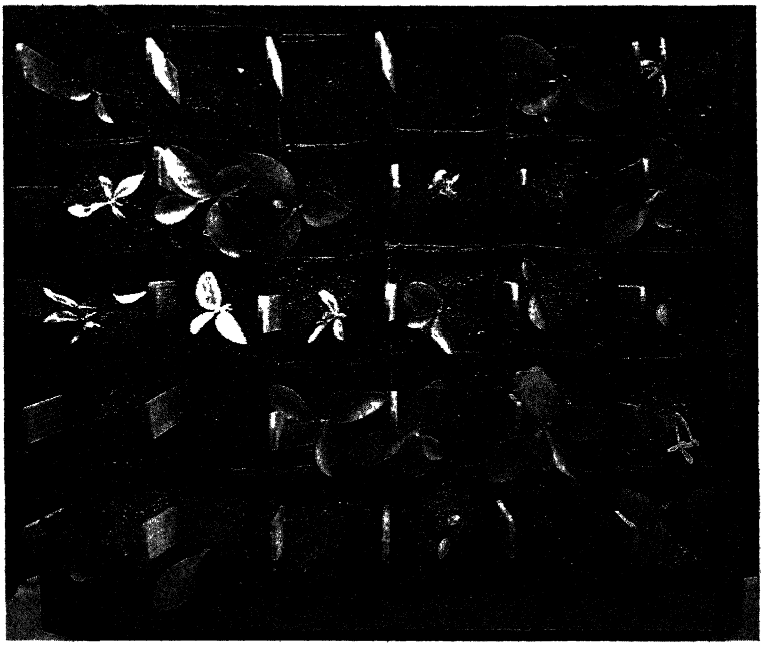

Fig. 1. Albinos among F_1 seedlings from the cross Paper-Rind (St. Michael) orange ♀ × Imperial grapefruit ♂, about 12 weeks after sowing. The albino leaves are small and in some cases already withering.

The large proportion of albinistic seedlings which may occur is illustrated by table 4. Reason will be given below for expecting higher proportions of apogamic progeny from selfing than are reported above for crossing.[4] In view of all the facts stated, it is very improbable that the albinistic seedlings are all or mainly extracted recessives *carrying the same gene for albinism.* Probably, in fact, many or most of them are produced apogamically. If this is the case, their abundance is very remarkable, especially when we consider the rarity of albinism and chlorophyll variegation as observed bud variations on orchard trees.

TABLE 4

ALBINISM IN A STOCK SEED-BED, FROM SEEDS OF ORDINARY ORCHARD FRUITS (*a*)

Type of seedling	Grapefruit		Sweet orange		Sour orange	
	Number	Per cent of total(*b*)	Number	Per cent of total	Number	Per cent of total
Green	176		173		237	
Whitish	16	7.4±1.2	16	7.2±1.2	4	1.6
White	24	11.1±1.4	33	14.9±1.6	4	1.6
All albinistic	40	18.5±1.8	49	22.1±1.9	8	3.3
Undetermined(*c*)	4		1		3	
Total	216		222		245	

(*a*) Blocks of seedlings systematically selected to avoid prejudice.

(*b*) The probable error is obtained as in table 2. With two exceptions, it is omitted with percentages under 10.

(*c*) Omitted from totals.

An infectious type of variegation, such as occurs in Abutilon (Babcock and Clausen, 1918, p. 381), is improbable in the present case, since the parent trees and (usually) the majority of the progeny are fully green. The albinism seems to be genetic, not pathological.

It may be worth while to suggest a provisional hypothesis for albinism. The recent demonstration by Demerec (1923) and Lindstrom (1924) of numerous genes for albinism in maize, together with the evidence for extensive heterozygosis in Citrus discussed below, suggests the possibility of high proportions of albinos among the sexual progeny. Further, somatic gene mutation in a tree heterozygous for albinism genes might often produce islands of albinistic tissue, and any embryo developed from these areas, either apogamically or sexually, would be albinistic. The great objection to the latter possibility is the scarcity of visible albinistic areas in older trees. Bateson (1919, 1921), however, has found that certain green-over-white periclinal chimeras do not give reversals of the relative position of their components, although the corresponding white-over-green chimeras do give such reversal; he suggests that differences in growth vigor may be concerned in this result. Possibly albinism often originates as a somatic variation in Citrus, although albinistic areas rarely develop far enough to be noticeable. It must be noted here that permanent green-and-white forms, evidently chimeras, do occur (Shamel *et al.*, 1920), and appear to have a mixed or mosaic condition of the apical meristem.

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[4] The consideration of the evidence (p. 388) for this expectation necessarily ignores the albinistic plants, which die before any other character than albinism can be determined.

We must keep in mind here the aberrant genetic phenomena so often associated with variegation (R. A. Emerson, 1922; Eyster, 1924).

Since the albinism is often only partial, and variegation so often shows genetic peculiarities, we cannot safely conclude that other characters are likely to be similarly variable among apogamic seedlings. Webber (1905), however, has reported remarkable variability among apogamic seedlings from interspecific crossing, finding in one case "5 or 6 different varieties" among 20 non-hybrid F_1 grapefruit seedlings produced by one cross. He concluded that such apogamic progeny appeared more variable than seedlings resulting from selfing. These variations seem to have related mainly to fruit characters.

Among the apogamic progeny from cross pollination that are now under observation at Riverside, genetic variations recognizable *in advance of fruiting,* aside from the "thick-leaved" form discussed below, and the proable case of albinism, seem to be very rare. The doubtful point here is the uncertainty whether a few variant individuals in our cultures are apogamic or not, but the evidence from crosses between very unlike forms indicates that these variants are usually hybrids, and therefore favors the interpretation just stated. On the other hand, among the apogamic progeny whose fruits have so far been studied in our cultures, several apparent cases of genetic variation have been observed. In the best-substantiated case, an old navel orange tree (N1 of table 3; not Washington), pollinated by grapefruit, has given apogamic progeny mostly with navel-marked fruit, usually seedless, and flowers destitute of pollen, but also including several trees that produce flowers with pollen and non-navel fruits with seeds. In general, so far, the apogamic progeny from selfing and those from crossing appear to be identical in type.

Genetic variation among apogamic progeny does not necessarily indicate the immediate agency of genic or chromosomal mutation, since the seed-parent tree may often be in a chimeral condition as the result of earlier genetic changes in somatic tissue.

It must be noted that the possibility of frequent variation among the apogamic embryos prevents strictly positive conclusions as to the proportion of sexual embryos, especially from selfing, and as to the genetic variability of the sexual embryos.

In this discussion, exception has been made of a thick-leaved form, showing no pollen-parent characters in crosses, which has frequently appeared in our cultures. This form, readily identifiable everywhere by the same general characteristics, has been found among the apogamic progeny of four horticultural varieties of sweet orange, three of grapefruit, four of *Citrus nobilis* (King, Dancy, Willow-Leaf, and Owari satsuma, and one of lemon. It often constitutes several per cent of the total number of progeny. As compared with ordinary apogamic seedlings of the same parentage, it is characterized by broad, thick leaves, stout shoots and thorns, somewhat lower vigor and slowness to bloom. Figure 2 shows, for three parent varieties, the differences in leaf form between normal and thick-leaved apogamic

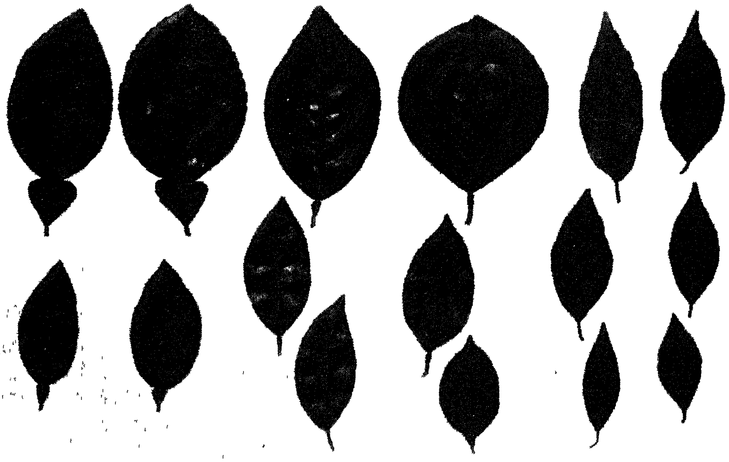

Fig. 2. Each vertical row of two or three typical leaves (one large, from a vigorous shoot, above, and one or two smaller below) represents a tree budded from a seedling. The leaves shown here and in the following figures, except as noted, were taken from trees that had grown two or three summers in the orchard. Figure 2 includes apogamic progeny only. First vertical row at left: seed-parent type from cross-pollination of Marsh grapefruit ("seedy strain"). Second row: thick-leaved type from same cross. Third and fourth rows: seed-parent and thick-leaved types, both from the same seed, from cross-pollination of Ruby orange. Fifth and sixth rows: the same for Willow-Leaf mandarin, both types from one seed.

progeny of the same parentage. Figures 3 and 6 give the same comparison for the thick-leaved and normal apogamic forms of Imperial grapefruit, as produced both by crossing and by selfing.

I have examined fruits from thick-leaved lemon (Lisbon), and a few from thick-leaved tangerine (Dancy), mandarin (Willow-Leaf) and orange (Paper-Rind and Ruby). In all these forms the oil glands of the rind appear larger than in the corresponding diploid apogamic progeny, and the surface of the rind has a characteristically coarser appearance. The acid content of the juice seems generally lower than in diploids. The lemon fruits seem approximately normal in juiciness and flavor, but the actual yield of juice in three tests was very low. The Dancy, Willow-Leaf, and Paper-Rind fruits were notably inferior in texture or flavor, or in both respects.

A doubled number of chromosomes ($n = 18$) has been reported for a thick-leaved form of orange (Frost, 1925*a*), and the thick-leaved form of grapefruit shown in figure 2 has recently been found to be tetraploid. Presumably, therefore, the other ten thick-leaved forms are also tetraploid. Since a thick-leaved and a normal apogamic seedling often arise from the same seed, it is probable that tetraploidy originates frequently, under the Riverside climatic conditions, in the nucellar tissue of Citrus species generally.

Muller (1925) has recently indicated the probable reasons why polyploid races originate much more readily in plants than in animals. Citrus seems to offer, in its development of embryos from single cells of somatic tissue, the most favorable general conditions possible for the origin of tetraploid individuals. Presumably tetraploid forms of Citrus have usually been eliminated, however, under both natural and artificial selection, by unfavorable tree and fruit characters. There probably has been little opportunity, therefore, for natural production of triploids from tetraploids, and seed reproduction of triploids would doubtless be hampered by a high degree of gametic sterility.

A few of our hybrids have characters suggesting triploidy. Presumably triploids and modified triploids can be produced by crossing tetraploids with diploids. If the horticultural disadvantages of tetraploids are generally absent from triploids, the production of triploids may become an important aspect of Citrus breeding, for several reasons. Triploids may be expected to be practically seedless. They *might* prove especially vigorous. There might also be advantage in the possibility of using a double dose of one parent type in the production of hybrids. And hybrid tetraploids should permit the production of triploid hybrids having, *on the average,* equal chromosome contributions from three ancestral races. Very frequent irregularities of chromosome reduction, as observed in pollen mother cells of thick-leaved orange (Frost, 1925*a*), may interfere seriously with the production of triploids.

Especially interesting breeding possibilities are suggested by Clausen and Goodspeed's (1925) tetraploid Nicotiana. This form, although derived from a sterile F_1 species hybrid having irregular meiosis, is fertile, evidently because meiotic pairing occurs, in the tetraploid, only between chromosomes derived from the same ancestral species, with consequent *complete homozygosis* and normal reduction. If interspecific Citrus hybrids will produce, asexually, tetraploids behaving in this way, these tetraploids should produce (aside from cytological accidents and new gene mutations) *gametes that are all alike.* Most of the progeny produced sexually by selfing such a tetraploid would presumably be indistinguishable from the progeny produced apogamically. In crossing, however, the possibility of using a hybrid *as a homozygous parent* might prove very useful. Even if the other parent were a highly heterozygous diploid, the number of possible F_1 types would be enormously reduced as compared with that resulting from the crossing of two such diploids.

Even with normal random reduction in a pure-species tetraploid, the recessive genes for which the plant is heterozygous should be largely "covered" by corresponding dominants in its gametes. These gametes should, therefore, represent the parental type much more closely, as a rule, than the gametes of the diploid form from which the tetraploid arose. If, however, that diploid form was highly heterozygous, the tetraploid may have more meiotic pairing between its *identical* chromosomes than between its *non-identical homologous* chromosomes. The normally produced gametes would then tend to represent their parent still more closely, the limiting case being the complete homozygosis that prevails when pairing regularly occurs between identical chromosomes.

This largely speculative discussion of the breeding possibilities of tetraploids seems to be justified by the slowness with which genetic data are obtained in Citrus, and the consequent especial desirability of formulating the problems with great care. The horticultural prospects of tetraploids seem to depend mainly on three factors: (1) the extent to which the tetraploid condition, and perhaps the triploid also, are in themselves inimical to desirable tree and fruit characters; (2) the meiotic behavior of tetraploids; (3) the possibility of obtaining tetraploids, especially homozygous ones, from diploid species hybrids.

HETEROZYGOSIS

Webber (1900*a*, 1905, 1906, 1907, 1912), Swingle (1910, 1913, 1913a) and Webber and Swingle (1905) have described and discussed the remarkable variability of F_1 species hybrids in Citrus. The salient features of the case may be stated as follows: (1) Various species of Citrus cross readily with each other and also (evidently less readily) with species of Poncirus (trifoliate orange) and Fortunella (kumquat); (2) these species usually seem to give, on selfing, only a moderate to slight amount of genetic variation; (3) F_1 hybrids between these species are remarkably variable, both in form and in vigor; (4) in the F_2 generation conspicuous segregation may occur, or the F_1 form may apparently breed true. Figures 3 and 4 illustrate the variability in size and form of leaves among the F_1 hybrid progeny from two species crosses, and figure 5 the variability in size and form of fruit in another cross.

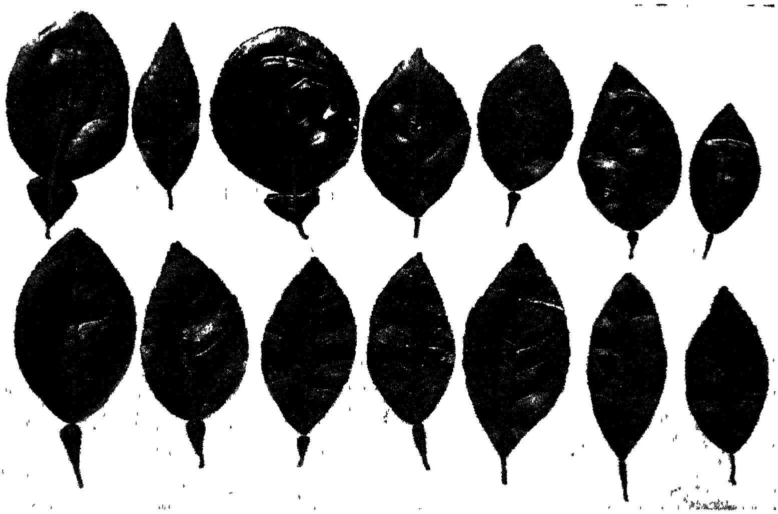

Fig. 3. Imperial grapefruit ♀ × Willow-Leaf mandarin ♂, F_1. One large leaf from each tree. First at left in upper row, seed-parent type (apogamic); second, pollen-parent type (apogamic, from a cross in which this variety is seed parent); third, thick-leaved type of grapefruit (apogamic); rest, hybrids, showing characteristic variation in form and size of leaves.

As Swingle (1913*a*) especially emphasizes, the F_1 hybrids may appear far more variable than either uncrossed parent species. To

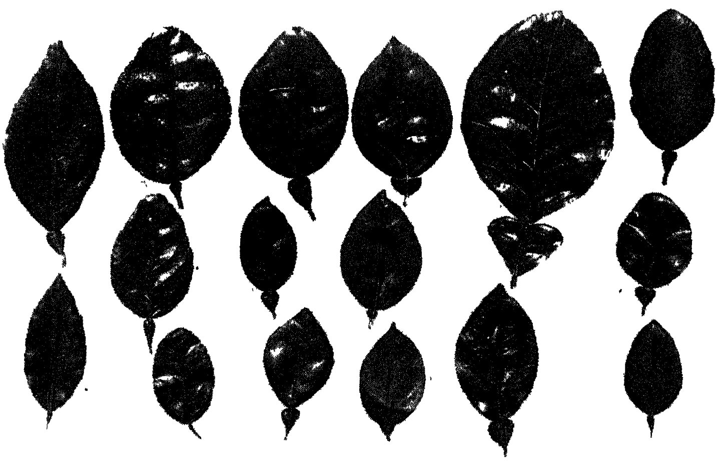

Fig. 4. Arrangement as in figure 2. Mediterranean Sweet orange ♀ × Imperial grapefruit ♂, F_1. Seed-parent type at left, the rest hybrids. (The pollen-parent type is shown in figure 3.)

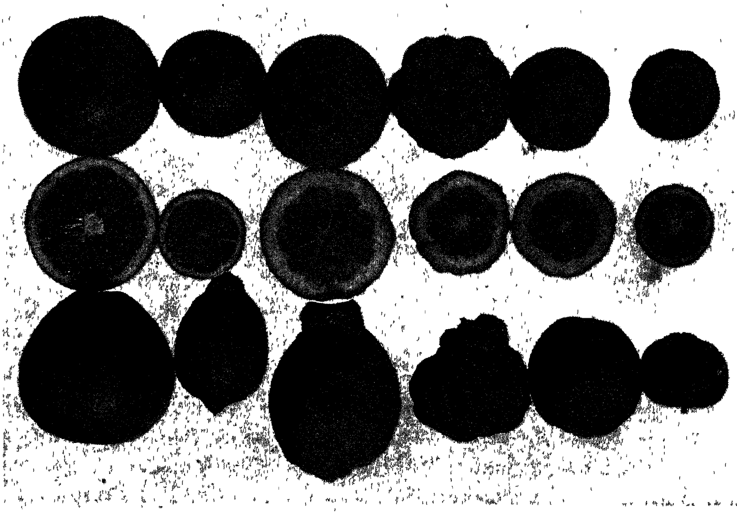

Fig. 5. Fruits, each vertical row from one tree. Imperial grapefruit ♀ × Lisbon lemon ♂. From left to right: seed-parent type, pollen-parent type (from reciprocal cross), and four hybrids.

explain this situation, he proposes the hypothesis of *zygotaxis,* which assumes "a positional or vectorial influence of chromosomes." That is, it is supposed that the relative effect of the individual chromosomes upon ontogeny depends to a large extent on their relative position in the nucleus, and that this position normally changes only at the time of fertilization. Adequate cytological support for this hypothesis is lacking, however, and the results of genetic experimentation seem unfavorable.

Sturtevant (1925) has recently secured from Drosophila certain evidence which seems to indicate that position may affect the potency of a gene. He concludes that two mutant genes of the bar series have more effect on development when they are carried in the *same* chromosome (as in double-bar, formerly called "ultrabar") than when they are in *homologous* chromosomes (as in homozygous bar). Another case, involving triploids, is similarly interpreted, the conclusion as to position of the genes depending in part on the fact that homologous chromosomes are "closely apposed" in somatic divisions.

Muller (1918) concludes that extensive heterozygosis in a pair of chromosomes may be expected to decrease their mutual attraction, and so to favor abnormalities of meiosis. Such abnormalities are often observed in hybrids from wide crosses. As Swingle (1913*a*) suggests, if the homologous chromosomes of the progeny of closely related parents tend to be associated in pairs in the somatic nuclei, that association may well be weakened or destroyed in interspecific hybrids.

The considerations stated in the last two paragraphs may be held to give some basis for the hypothesis of zygotaxis. At best, however, it seems seriously inadequate as the main explanation of the Citrus phenomena in question. If a pairing attraction is weakened in species hybrids, there is no obvious reason, cytological or genetic, to expect that many definite and distinct chromosome configurations, permanent throughout somatic life and with extremely marked effects on the relative potency of genes, will be established. The *accidental* formation of somatically permanent chromosome configurations at fertilization seems especially improbable; therefore, if many such different *permanent* configurations should occur among the zygotes from one cross, it would seem that they must depend on genic differences among the gametes which united to form those zygotes—or, in other words, *on extensive heterozygosis of the parents.* If, however, the parents are thus heterozygous, the genic differences among the progeny will probably account directly for at least the major portion of their somatic variability.

Such cases as that of beaded wing in Drosophila (Muller, 1918) show that recessive genes may often be suppressed by linkage with other genes which have a recessive lethal effect, and that selfing or inbreeding may fall far short of revealing all the genetic potentialities of an organism, as represented by its genic constitution. It is also generally conceded that the effect of a particular gene may be greatly modified by differences in other pairs of allelomorphs. It is now evident that heterozygosis offers almost unlimited possibilities of F_1 variability, and therefore the hypothesis of zygotaxis seems to meet no serious general need in genetic theory.

It may fairly be assumed, therefore, that the striking variability among F_1 Citrus hybrids is mainly due to heterozygosis of the parent forms. Evidence bearing on this hypothesis will now be considered. Variation in chromosome number may be concerned in some cases, but this seems very improbable as a regular source of such extensive series of forms.[5]

A. C. and A. L. Hagedoorn (1914) have suggested that Citrus varieties are highly heterozygous but self-sterile, and that when not cross-pollinated they reproduce by apogamy alone. The evidence now indicates, however, that self-sterility is not concerned, and that the *viable* progeny from selfing, although more largely of apogamic origin than in the case of crossing, are often not exclusively so.

Coit (1914) states that the evidence indicates that cross-pollination is unnecessary "in all naturally fertile varieties of orange." Ikeda (1904) reports that cross-pollination between certain varieties of orange results in failure to set fruit. My observations indicate that, in Citrus varieties with good pollen, seeds are set about as readily in selfing as in crossing. That this situation is not usually due to apogamy in the absence of fertilization, is indicated by evidence already presented (p. 371).

There is also direct evidence for the occurrence of segregation with selfing. Swingle (1910) reports that some F_1 citranges "reproduce almost exactly the parental type" in their progeny, while with other citranges part or all of the progeny show typical F_2 variability, ranging nearly from one P_1 species to the other. Evidently the *viable* embryos are all produced apogamically in the first group, but not in the second. Similar evidence from selfing of commercial varieties of Citrus has been obtained in our cultures. As a specific illustration, among 122 seedlings from 99 seeds of selfed Imperial grapefruit, 13 young trees show marked differences from the usual type of the variety (fig. 6); 7 of these belong to the thick-leaved type and are doubtless apogamic in origin (p. 380), but the 6 others probably represent as many distinct recombination types.

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[5] Longley (1925) has recently found that two Citrus hybrids, one of them intergeneric according to Swingle's (1914) classification, have the normal number of chromosomes ($n = 9$).

When F_1 cultures from selfing and from crossing are compared, there is in general a remarkable parallelism. We commonly find (aside from the thick-leaved type) two very distinct groups of progeny, which occur in interspecific crosses, in intraspecific crosses (as between two varieties of sweet orange), and with selfing. One group consists of trees essentially identical with the seed-parent variety, with occasional differences such as Webber (1905) has discussed, while the other group, often relatively small, consists of marked variants. In the case of crossing, the former group plainly is entirely of apogamic origin, while trees of the latter group usually show clearly the influence of the pollen parent. That the former group is mainly or entirely of apogamic origin in the case of selfing also, seems highly probable; if not, the parent trees must usually breed true to a very remarkable extent with self-fertilization, while producing extremely wide variation with cross-fertilization.

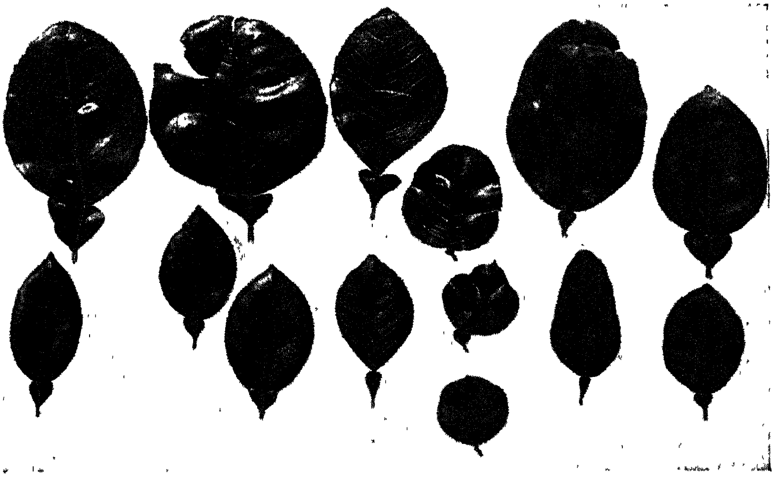

Fig. 6. Arrangement as in figure 2. Progeny of selfed Imperial grapefruit. The three vertical rows at the right are from two-year trees in nursery rows. First at left, seed-parent type; the rest, variant types (second, thick-leaved type, from same seed as first tree; fourth, a small, weak type).

We have only about 600 progeny from selfing; none of these are in the cultures of 1917 which give our best hybrid data, and very few of them come from the parent varieties with few adventitious embryos. The available evidence is therefore meager. It strongly suggests, however, that sexually produced progeny are generally rarer with selfing than with crossing, and much less vigorous. Lisbon lemon has given 9 progeny from selfing, of which 4 appear to be identical with the apogamic progeny from crossing. The other 5 progeny are all markedly variant, and range from trees considerably inferior to Lisbon in vigor to ones so feeble that it is difficult to keep them alive. It seems plain that the latter group corresponds to the hybrids in the cultures from crossing. Similarly, selfed King, among 38 progeny from 38 seeds, has given 29 trees all evidently typical King, 1 thick-leaved, and 8 other variants. In this case fruits from nearly all the trees that closely resemble the parent variety have been examined, and these trees all seem to be identical in type with apogamic progeny from cross-pollination of King. The variants from selfed King, besides being much less numerous than the King-like progeny, are all or nearly all inferior in vigor (at least 6 of the 8); on the other hand, the hybrids in the corresponding lots from cross-pollination constitute more than half of the total progeny, and are usually similar to their apogamic sibs in vigor. The progeny of selfed Imperial have already been discussed. Part of the other selfed varieties (Paper-Rind orange, Ruby, Willow-Leaf) have given (besides occasional "thick-leaved" progeny) a very small proportion of conspicuous variants, always weak, and several varieties (some in very small cultures) seem to have produced apogamic progeny alone.

In the causation of inviability, albinism (pp. 377–379) may be of much importance.

Evidently, then, ordinary Citrus varieties, as well as many F_1 hybrids (Swingle, 1910) reproduce mainly or very largely by apogamy when selfed. Selfing probably produces, as a rule, fewer and weaker viable sexual progeny than does crossing. This situation has an important bearing on the variability of stock seedlings (Webber, 1920). Probably most of the undesirable variant types among nursery seedlings are produced by fertilization. Citrus clones which give genetically uniform seedlings from selfing are evidently not ones which "breed true" in the ordinary sense, but ones which reproduce almost entirely by apogamy. From this point of view, clones which produce seeds with fairly numerous embryos are likely to give better results than clones with usually monoembryonic seeds. The suitability of the Florida Rough lemon for use as a stock plainly depends partly on the fact that it is highly polyembryonic, and therefore, unlike the Lisbon lemon, reproduces mainly by apogamy when selfed.

The F_1 hybrids from a Citrus cross often vary greatly in vigor, as well as in morphological characters (fig. 7). Forms conspicuously lacking in vigor are often produced. Crandall (1922) has reported similar results with interspecific crosses of apples, and Dr. M. J. Dorsey, on examining some of our Citrus hybrids recently, stated that they seemed less variable than hybrids between certain plum species. Wellington (1924) has reported that numerous varietal crosses of apples have usually produced some weak types among the F_1 progeny. He ascribes this result, together with the great variability of fruit characters, to extensive heterozygosis of the parent clones. Where *all* the hybrids are feeble, as in some apple crosses, it may be inferred that the genetic reaction systems of the parent species are *in general* too unlike to permit normal development in their hybrids (Goodspeed and Clausen, 1917). When, however, some F_1 hybrids are vigorous and some feeble, it may be inferred that the parents are heterozygous and that *some progeny combinations of genes* are markedly more favorable than others. With maize (Jones, 1918) the unfavorable combinations usually seem to be homozygous recessives, since selfing usually decreases vigor and increases the proportion of abnormal recessive types. In view of the predominance of unfavorable recessives among the mutations of Drosophila (Muller, 1918), and the probability (Sturtevant, 1921) "that closely related species have many genes in common," the unfavorable combinations in species crosses of Citrus may be, in large part, merely cases of absence of various favorable dominant genes that are heterozygous in the parents.

In the apple and the plum, extensive heterozygosis is favored by widespread self-sterility, and also, if mutation occurs in somatic cells, by the long life of the individual; it is therefore to be expected that crosses, whether varietal or specific, will give highly variable F_1 populations.

In Citrus, self-sterility does not seem to be concerned, but the long-life factor[6] is present and the frequent bud variations suggest, although they do not demonstrate (Clausen and Goodspeed, 1923) the occurrence of gene mutation. The conditions therefore seem to be favorable (Muller, 1918; Jones, 1918) for the accumulation of unfavorable recessive genes. Moreover, the long-life factor is markedly reënforced by apogamy. A Citrus race might be heterozygous for many recessive genes that are sublethal or lethal when homozygous, and yet reproduce by apogamy for an indefinite period.

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[6] The word *factor* is used in its general sense in this paper; in the sense of "genetic factor," *gene* is employed exclusively.

The conditions in Citrus are also favorable for the development of heterozygosis by crossing, since the flowers are attractive to insects and produce an abundance of pollen, while (among the true Citrus species) there seems to be no interspecific sterility to impose limitations on wide crossing.

The evidence on heterosis[7] in Citrus is also in accord with the assumption of extensive heterozygosis. It seems probable that ordinary Citrus varieties are complex heterozygotes, whose unfavorable recessive genes cause a great and general decrease of vigor on selfing, and frequent decrease of vigor even in species crosses.

In our cultures, hybrids decidedly exceeding in vigor the more vigorous parental type, as represented by its apogamic progeny, seem to be exceptional, while feeble, slow-growing hybrid forms are common in some crosses (fig. 7). Marked heterosis, such as Webber and Swingle (1905) report for some of the citranges, seems at first sight to be unusual in these crosses. It must be noted, however, that apogamic embryos do not furnish a satisfactory standard of comparison for the estimation of heterosis in hybrids; the proper standard is obviously given by the sexual embryos produced by selfing.

It has been shown that sexually produced progeny seem to be decidedly more numerous with crossing than with selfing. This conclusion agrees with the expectation that cross-fertilization will produce the more vigorous embryos, more often able to withstand the competition of those produced by apogamy.

It seems significant that Swingle (1910) finds such marked vigor, even as compared with the parental forms, in citranges, which are to be considered intergeneric rather than interspecific hybrids; as would be expected, F_2 citranges and back crosses with the orange are less vigorous. Further, Swingle reports that crosses of F_1 citranges with the grapefruit, which belongs to a species markedly different from the orange, or with the kumquat, representing a third genus, yield still more vigorous progeny. Thus it appears that as a rule the hybrids from the widest crosses are decidedly the most vigorous. If we could adequately compare hybrids between and within species of true Citrus (as Swingle delimits the genus) with sexually produced progeny from selfing of the same species and clones, presumably we should find much heterosis even in these hybrids, and far more evidence of selective elimination with selfing than with crossing. In general, we may conclude that, with Citrus and its near relatives, the expression of unfavorable genes among the progeny decreases with distance of parental relationship. This heterotic effect probably increases in most cases to the limits of possible crossing, without being overcome by any unfavorable effect of the genic unlikeness of the parental forms.

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[7] The theory of heterosis, or hybrid vigor, proposed by Jones (1918) is here accepted as the best general explanation of this phenomenon.

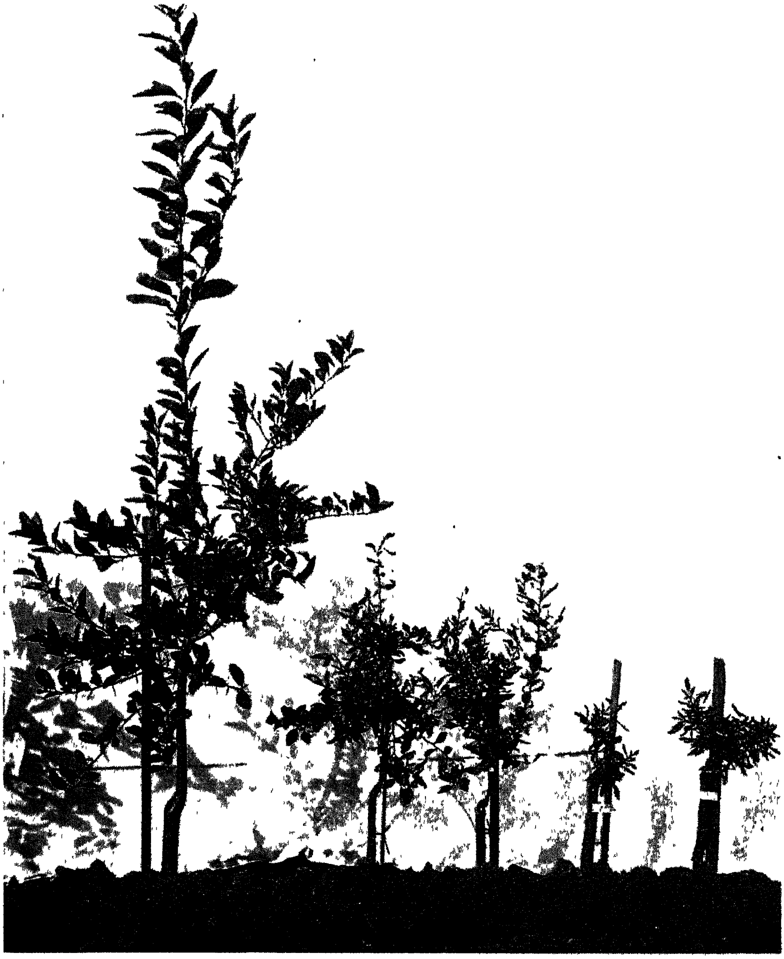

Fig. 7. Ruby orange ♀ × Valencia orange ♂, F_1. Two-year budded trees in nursery. First tree at left, normal orange type resembling parents, probably apogamic; the rest, two dwarf types, each budded in duplicate.

Obviously, lethal and sublethal effects in selfing and crossing of Citrus may not be entirely the result of homozygosis of inevitably unfavorable genes, but may be in part a result of "incompatible" recombinations (Goodspeed and Clausen, 1917). In any case, the wide differences in vigor among progeny from the same parentage indicate complex heterozygosis of the parents.

When we consider the fertility of the F_1 hybrids, we find wide variability, similar to that with respect to vigor. The sterility shown by certain hybrids seems to be, in large part at least, a matter of individual genic composition. Thus Swingle's citrange evidence cited above indicates that these F_1 generic hybrids are sometimes highly fertile when selfed, and sometimes nearly or quite sterile (so far as viable embryos are concerned), aside from apogamic reproduction. This case therefore differs from that of certain Nicotiana species hybrids reported by East (1921), since the latter regularly show a high degree of sterility in the F_1 generation. This difference may well be due to a condition of complex heterozygosis in the Citrus species concerned.

The evidence so far discussed indicates that the apparent "breeding true" of selfed Citrus varieties, which naturally suggests homozygosis, is due primarily to a predominance of apogamic progeny, which seems to be usually much greater here than in species crosses. Further, probably many genes that come to expression in hybrids are usually or always suppressed in selfing. Finally, the appearance of uniformity is in part illusory, since, outside of special genetical cultures, occasional weak individuals are unlikely to come to fruiting, or to be noted at all without special search.

CHIMERAS AND BUD VARIATION

For centuries Citrus has been noted for striking somatic variations, especially for variant sectors in the rind of the fruit. Certain "bizzarria" forms, such as the one which Risso and Poiteau (1818–22, pl. 52) describe under the name "bigaradier bizarrerie," with fruits combining characters of two or three species, were attributed by two sixteenth-century writers, Porta and Nato (Savastano and Parrozzani, 1911), to development of sprouts from graft or bud unions.

Shamel and his associates (1912, 1918, 1918*a*, 1918*b*, 1920, 1920*a*, 1923, 1924, 1925) have shown that bud-variation forms in Citrus are somewhat numerous and of considerable agricultural importance. Modern genetic theory provides three possible general explanations of the origin of such variations—gene or point mutation, chromosomal duplication and deficiency (whether involving whole chromosomes or limited sections), and loss and rearrangement of components in chimeras. Eyster (1924) has perhaps added a fourth in his hypothesis of qualitative mitotic division of certain genes. That is, while such a process would be included among the probably "diverse processes" (Sturtevant, 1925) of intragenic change, or mutation, of which we know so little, it seems to be essentially distinct from the fundamental changes which we surmise to supply the ultimate material of evolution. Eyster's intragenic units might, however, be merely labile "side-chains," subordinate elements in a single complex structure (the gene), and not the coördinate components of a compound genic structure.

R. A. Emerson (1922) has comprehensively discussed the origin and nature of bud variations. Coit (1915) has shown how chimeras may result from the occurrence of mutation in somatic tissues of Citrus. Clausen and Goodspeed (1923) have well presented some fundamental morphological considerations relating to chimeras, and pointed out the extreme difficulty of detecting the occurrence of gene mutation in such cases as that of Citrus.

It should be fairly easy to test the possibility of chromosomal mutation in forms that produce pollen, since Belling's (1921) iron-acetocarmine method can be used with Citrus (Longley, 1925; Frost, 1925; 1925*a*). The fact that bud-variation forms of Citrus often differ decidedly from the parent race in various characters, seems favorable to the possibility of chromosomal mutation. Little work seems to have been done anywhere which bears directly on the causation of bud variation in Citrus, although the evidence from other plants (Winkler, 1910; Bateson, 1916, 1919, 1921; Clausen and Goodspeed, 1923) suggests that chimeral phenomena may be of much importance.

If Citrus forms are extremely heterozygous, somatic gene mutations, if they occur, will relatively often come to somatic expression (Muller, 1918). Whether such heterozygosis favors abnormal somatic mitosis seems to be entirely in doubt. R. A. Emerson (1922) found that a variegation gene in maize "mutates" more frequently when combined with an allelomorph for white than when homozygous, and Eyster (1924) suggests an explanation based on his hypothesis of heterogeneous structure of the gene concerned. We might expect, therefore, that the average instability of any such genes present in Citrus will tend to be increased in the presence of extensive heterozygosis.

A bud-variation type, whatever its cytological basis, presumably originates in a single cell. If the variation depends on non-disjunction, either of whole chromosomes or of smaller units of chromatin, twin daughter cells may carry two different and complementary new types (Eyster, 1925). If the variation involves some change occurring within a single gene according to the current conception of gene mutation in the narrower sense, a single new type is produced.

If a variant cell occurs and its descendants persist in the apical meristem of a shoot or bud, further development consists, for a time at least, of two kinds of tissue, and the shoot has become a chimera. Doubtless many variant initial cells are too deficient in vigor to compete successfully with normal cells, so that only a part of the new types formed ever come to dominate even one bud. Probably many twin variations are never recognizable as such, because of early elimination, either selective or accidental, of one of the two complementary types. This consideration increases the probability (R. A. Emerson, 1922) that unequal mitosis is the predominant cause of the origination of bud-variation types. It is interesting to note here that the bud-variation strains described by Shamel and his associates (1918, 1918*a*, 1918*b*, 1920, 1920*a*, 1923, 1924, 1925) seem generally to range from moderately less vigorous to considerably more vigorous than the parent variety.

At an early stage of the process described in the last two paragraphs, the shoot affected is an incomplete periclinal chimera, since the new type constitutes a sector in the cell layer or layers to whose formation the variant initial cell contributes. What happens later must depend on the spatial regularity and uniformity of the meristematic cell divisions, and on the location of new buds. There is reason to believe that Citrus chimeras are often relatively unstable in the relations of their components.

Sectorial fruit chimeras are frequent in Citrus (Coit, 1915; Babcock and Clausen, 1918; Shamel *et al.*, 1918, especially plates). Frequently a longitudinal sector differs from the rest of the fruit in thickness or color of rind. In some cases a whole tree shows so marked a tendency to the production of variant fruits that it appears to be in a chimeral condition throughout.

Sometimes a fruit has two adjacent sectors, of similar width, whose rind varies in opposite directions from the normal condition. This may be considered definite evidence (Eyster, 1925) that the variation is due to differential mitosis, perhaps to non-disjunction of chromosomes. The paired sectors may be unlike either in color or in thickness of rind, or in both at once. If non-disjunction of whole chromosomes is not involved in all such cases, Eyster's hypothesis of qualitative division of individual genes may apply, in Citrus, to genes other than those especially determining color. In fact, there seems to be no reason to suppose that such a process, if it occurs at all, is confined to "color" genes, although it might seldom be discoverable in other cases.

The corrugated strain of navel orange described by Shamel *et al.* (1925) may be an unstable periclinal chimera. Coit (1915) has described a similar case.

A Valencia orange tree in one of our experimental plots has one large branch of a distinct type, which regularly produces seedless fruits with corrugated rind. If this branch is a chimera, it must be periclinal, and relatively stable. We have another form, derived from a variant branch of Valencia, selected by Shamel[8] for its corrugated rind, which seems to be a mixed chimera ("hyperchimera" of Winkler) of a peculiar kind. Some fruits are normal in appearance, but scattered among these are some which are completely and heavily corrugated. Many fruits are intermediate, ranging from nearly normal to much corrugated. In this case the usual visible variation among fruits is not, as in the case of the Golden Buckeye navel orange which is discussed in the next paragraph, in the relative superficial proportions of two separate components of the rind of the same fruit, but relates to the rind of the whole fruit. Some intermediate fruits, for example, have a smooth rind with broad, shallow ribs, while others show general but slight or moderate true corrugation. That this variation is not due to general physiological factors acting on a readily modifiable type, is indicated by the magnitude and generality of the variation, and is practically proved by one fruit which had sharply contrasting segments of normal and corrugated rind. We may surmise, therefore, that the intermediate fruits are periclinal chimeras in which the number of cell layers of the outer component varies, doubtless largely as a result of similar variation in the apical meristem of the young flower buds. Possibly, as Eyster (1924) assumes for variegation, an unstable gene is involved.

The case of the Golden Buckeye navel orange (Shamel *et al.*, 1925) is also of especial interest here. The rind of the fruit is more yellowish and thinner than that of the Washington navel orange, with knobs, stripes, and wider sectors of thicker, rougher, more reddish rind, resembling that of Washington. Somewhat frequently, however, on our trees, a branch produces fruit having only the deeper, more reddish color of Washington.[9] In such cases the rind characteristics are like those of Washington; the "navel" is usually more conspicuous than in Buckeye; and the shape of the fruit changes, probably in part, at least, because of the greater development of the navel structure. The fruits on these variant branches seem, in fact, to be indistinguishable from those of Washington. Apparently the Golden Buckeye is a mixed or mosaic chimera, of which one component resembles Washington while the other is similar to the Golden Nugget (Shamel *et al.,* 1918). If it is a periclinal chimera, evidently the inner component must emerge with remarkable frequency. It may be worth noting here that the typical commercial Golden Nugget is a dwarf form, and that plantings of this variety are, according to Shamel, always mixed with standard-sized trees, possibly of bud-variation origin.

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[8] Mr. Shamel has kindly given me permission to publish this description.

The Thomson orange (Shamel *et al.*, 1925) often gives rise to *several* other types of navel orange, and not simply to one type from which Thomson arose by bud variation. This case and other similar ones suggest that marked genic instability is an important factor in the situation with these forms. It does not seem likely that all these variations are produced by changes in chromosome number followed by chimeral phenomena, although some trees may well be complex chimeras.

Probably all of the three types of Citrus "bizzarria," described by Savastano and Parrozzani (1911) as natural hybrids, are chimeras, not interspecific hybrids. These authors mention frequent color chimeras in the fruits (see their plate 1). The great variation in sugar and acid, both between trees and on the same tree, shown by the only form extensively studied (orange-colored lemon, "limone aranciato"), plainly indicates a general chimeral condition, with the relationships of the two components decidedly variable. Forms like these, which clearly combine the characters of two or three species, are best explained by Porta and Nato's graftage hypothesis (p. 392). Chimeras due to graftage may be called *synthetic* chimeras. On the other hand, chimeras which arise as a result of genetic variation within a clone may be called *autogenous.*

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[9] I have seen at least three or four such branches on two rather small trees.

Cavara (1912), after mentioning Savastano and Parrozzani's forms and their use of the word *chimera* for fruits of mixed type, described a tree with ribbed or corrugated fruits, which bore several branches with smooth-rinded fruit. He concluded that the tree was most probably a chimera resulting from graftage, but it may well have been an autogenous chimera.

Trees which merely produce occasional variant shoots or fruits may in some cases be periclinal chimeras throughout, like Winkler's (1910) solanaceous chimeras, and the apple chimera described by Stout (1921). The Citrus chimeras, however, are doubtless usually autogenous, while Winkler's forms, at least, are synthetic. Thus many of the observed instances of bud variation in Citrus may be merely the result of irregularities of growth in long-existent chimeras.

If chimeras are very common in Citrus, they may largely explain the genetic differences that occur among the apogamic progeny of the same parent tree (p. 379). Little "islands" of variant tissue, which might never come to dominate their respective branches, may often give rise to apogamic seedlings that are visibly unlike the parent.

SUMMARY

This paper reports experimental results bearing mainly on the genetic significance of apogamy in Citrus. It also attempts a general evaluation of the published evidence relating to Citrus genetics. The data and discussion may be summarized as follows:

1. Polyembryony occurs generally in Citrus; adventitious embryos develop by proliferation of cells surrounding the embryo sac. It is here shown that the embryos are often much more numerous than the resulting seedlings, and that horticultural varieties differ greatly in characteristic amount of apogamy. This last fact seems to be important in connection with the choice of clones for the production of nursery stocks.

2. Interspecific and intergeneric crosses involving Citrus species exhibit, aside from the apogamic progeny, remarkable variation in the F_1 generation, suggesting an extremely heterozygous genetic constitution in the parental forms. Most seedlings from selfing are closely similar to the parental clone.

3. Fertilization seems to be usually necessary for the initiation of apogamic development. The sexually produced embryo is, however, frequently eliminated by the competition of apogamic embryos. Data

here presented indicate that the sexual embryo is more often eliminated in clones in which apogamic embryos are especially abundant. This fact should be considered in planning Citrus hybridization.

4. Doubtless the elimination of sexual embryos is often highly selective, largely because of frequent development of homozygosis of unfavorable genes. It is to be anticipated, therefore that selective elimination in favor of the apogamic embryos will tend, in general, to be most severe with selfing, and least so in relatively wide crosses. Some evidence presented indicates that the sexual progeny from selfing usually are both fewer and weaker than those from crossing.

5. Some especially "wide" crosses show marked heterosis when the parental types, as represented by progeny produces asexually, are taken as the standard of comparison. By the proper standard (sexual progeny resulting from self-fertilization), with consideration of viability as well as of relative vigor of viable plants, the favorable effect of crossing is presumably general and great.

6. It is suggested that the occurrence of apogamy in Citrus has favored the development, perhaps by mutation, of a very complex condition of heterozygosis, probably including lethal and sublethal genes, in Citrus forms generally. Crossing may have produced or contributed to this result, but its agency need not be considered essential.

✓7. Bud variations apparently affecting whole branches are frequent in Citrus. Sectorial chimeras are common, and evidently periclinal and mixed chimeras also.

8. The numerous bud-variation forms of Citrus presumably originate in single cells, either by gene mutation or by differential mitosis. In the former case, at least, their somatic expression is doubtless favored by the presence of numerous heterozygous recessive genes. The production of recognizable bud variations then requires bud formation in an area of variant tissue, and may often be due to irregular tissue development in periclinal chimeras. The abundance of bud variations with some Citrus forms apparently depends upon a permanent chimeral condition of the types in question. Some of the bud variations of Citrus suggest a special genic instability, perhaps fundamentally unlike typical gene mutation, such as has been postulated for cases of variegation.

9. In addition to the *autogenous* chimeras just mentioned, *synthetic* chimeras, resulting from graftage, evidently occur in Citrus.

10. The remarkable variations which sometimes occur among apogamic seedlings may be partly due to chimeral conditions in the

parent trees. A pollen-sterile navel orange has produced apogamically several fertile non-navel progeny.

11. A "thick-leaved" apogamic form is described, which has been produced by four species and twelve horticultural varieties. It has been shown in two cases to be tetraploid, and presumably is so in general. It may be valuable as a means of producing triploid hybrids.

12. Some evidence is presented on seedling albinism; its frequent production by some parents may be due primarily to heterozygosis for various genes for albinism, and perhaps to the presence of unstable genes such as occur in various cases of variegation in other plants.

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THE EFFICACY OF LEAD ARSENATE IN CONTROLLING THE CODLING MOTH

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The investigator who undertakes an orchard spraying experiment for the purpose of determining the value of one or more elements in the spraying method of controlling the codling moth, *Carpocapsa pomonella* (Linn.), is confronted by a large number of unknown and uncontrollable factors which influence in various ways and degrees the results of his experiment. Conclusions arrived at under such conditions of experimentation are likely to be lacking in explicitness and accuracy, and they may sometimes be wholly erroneous. It is a well known fact that the vast literature on codling moth spraying is replete with contradictory experimental data, and discordant opinions and beliefs. A number of points of primary importance regarding this method of control have continued to remain largely in the realm of assumption and theory every since spraying was first employed in codling moth control nearly fifty years ago. Spraying tests have been confined almost without exception to orchard conditions.

While assisting in some orchard experiments during the summer of 1917 to determine the relative merits of spraying and dusting with lead arsenate as a means of controlling this pest, I was impressed by the fact that so many variable factors were encountered as to render the resultant data of very doubtful value. Similar experiences met with in subsequent investigations and a study of the investigations of other entomologists emphasized the desirability of more accurate experimentation than could be accomplished under orchard conditions. It was during the late summer of 1920 that the idea was conceived of performing experiments with freshly hatched codling moth larvae in the laboratory. The work started that year was continued in a limited way in 1921, while fairly extensive studies were made in 1923 and 1924.

The earlier work was conducted at the Idaho Experiment Station; that of 1923 was performed in the laboratories of the Golden State Milk Products Company of San Francisco, and that of 1924 at the University of California. This paper* is based chiefly on the investigations of 1924.

Probably no other insect in the history of American horticulture has been the subject of more discussion and experimentation than the codling moth. The literature relating to it is very extensive. In 1888, L. O. Howard[9]† stated that the Office of Entomologist of the United States Department of Agriculture had proposed compiling a list of American writings on the codling moth, "but soon found that it would consume altogether more space than could be allowed." It is probable that the literature has increased several fold since that year.

Much of the published matter on the use of arsenical compounds in spraying for codling moth is perplexing to the reviewer. It is sometimes impossible to determine whether the statements of a writer are based on his own observations and experiments or on those of other persons, or whether they are based merely on assumptions. There is much contradictory and inconsistent data, explanations for which are either obscure or entirely lacking.

In order that the reader who is not especially familiar with the subject may have a better understanding of the significance of the research reported herein, a brief review of the history of the spraying method of control is given. This review is of necessity very fragmentary. Only a few references out of hundreds have been cited.

BRIEF HISTORICAL REVIEW OF CODLING MOTH SPRAYING

Early Experiments with Arsenical Sprays

The use of arsenical compounds in codling moth control originated in 1878 when it was discovered that the practice of spraying apple trees with Paris green to destroy the spring canker worm not only controlled that pest but also reduced the injury from codling moth.[1] Experiments on the value of this treatment, made by Cook of Michigan in 1880, indicated that it was highly effective.[2] Other experiments reported in 1886 by Forbes of Illinois[3] and Goff of New York[4] confirmed the findings of Cook. Since then practically all investigations of control have dealt either directly or indirectly with the arsenical method. Soon after 1890 all authorities seem to have reached the conclusion that spraying was so efficacious that the old methods of control, including banding of trees and destroying wormy apples, were no longer worth while. Since the establishment of the State Agricultural Experiment Stations in 1888, spraying experiments have been carried on more or less continuously in every important apple-producing state in the United States. Something over two hundred state and federal bulletins and major papers in serial publications, containing original reports of such experiments, have appeared during the past twenty-five years.

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* Acknowledgment is gratefully made for suggestions and assistance given by members of the staff of the Division of Entomology and Parasitology of the University of California at Berkeley and to Mr. C. E. Gray, president of the Golden State Milk Products Company, for permission to use the photographs shown in figures 7, 8, 12 and the lower part of figure 2, which were made by me in the laboratories of that company.

† Superior figures refer to bibliography at the close of this paper.

POTENCY OF THE SPRAYING METHOD

After eight years of experimentation in spraying with Paris green, Cook, in 1888, came to the conclusion that if all apples of a tree "received the poison" no injury from codling moth would result.[8] Wormy apples, he believed, could be attributed only to "lack of thoroughness" in applying the spray. In general agreement with Cook, later authorities have inclined to the belief that if every calyx and every apple were thoroughly treated with arsenical spray, practically complete destruction of codling moth larvae would be accomplished. Some writers have been very emphatic in statements to this effect.

Instances of unsatisfactory control have been attributed chiefly to two factors: first, failure to spray at the most suitable time or times during the season and, second, lack of thoroughness in applying the spray. The object of nearly all codling moth investigations of the past quarter century has been to obtain more accurate information on the "timing" of spray applications, and on ways and means of securing greater thoroughness. The former has dealt with life history studies of the insect while the latter has been concerned chiefly with spraying machinery and equipment.

SPRAY APPLICATIONS

Spray applications may be classified in general as of two types, the calyx spray and the cover sprays. The first refers to spraying just after the petals of apple blossoms have fallen. The object of this application is to place a quantity of poison in the calyx cavity of the developing fruit before the cavity becomes closed by the infolding of the sepals. The cover sprays are those that are applied after the calyx application, the purpose being to place a covering of poison over the surface of the growing apple.

NUMBER OF SPRAY APPLICATIONS

Forbes[6] and Cook[8] early arrived at the conclusion that only the calyx spray and the cover sprays applied soon after the calyx spray were of especial value. This position was strongly supported by Card[31] and Slingerland.[32] On the contrary some contemporary authorities, including Lake,[12] Washburn,[27] and Cordley,[30,41] regarded the calyx spray of little value and emphasized the importance of the later cover sprays. In 1900 Aldrich[36] reported experiments which indicated that the calyx application was over five and one-half times as effective as the cover sprays. Simpson[40] and Gillette[39] reported that eighty per cent of the first brood larvae entered at the calyx. Similar evidence was presented about that time by a number of other investigators and, as a result, there developed a renewed interest in what later became known as the "one-spray method" of codling moth control, or control by the calyx spray only.

The numerous and extensive investigations that were carried on during the period from about 1905 to 1915 centered largely around the one-spray method of control. Considerable data was published indicating that the calyx spray was all-sufficient. It is of particular interest to note that Gillette,[46] Weldon,[47] and Melander[48] reported evidence from the western states which tended to show that the greater the number of sprays applied, the less effective was the control. Melander gathered data from upwards of one hundred apple orchards in Oregon and Washington for the years 1909 and 1910, which showed approximately one per cent wormy apples where the calyx spray only was applied, four and one-half per cent where the calyx and one cover spray were applied, four and one-half per cent for the calyx and two cover sprays, and eight per cent where the calyx and four or more cover sprays were applied. Quaintance,[49] after referring to eleven experiments in eight different states, remarked that "the results of the one-spray method are on the whole excellent and fairly uniform."

TIMING OF SPRAY APPLICATIONS

The rise and subsidence of the one-spray method constitutes an interesting chapter in the annals of codling moth control. There were many marked cases of failure but proponents of the method contended that unsatisfactory control was due to the spray not being properly applied. By about the year 1915, however, nearly all authorities seem to have reached the conclusion that the pest could not be satisfactorily controlled by means of the calyx spray alone, excepting, perhaps, in restricted parts of the country where, because of peculiar climatic conditions or other factors, the codling moth had failed to become abundant or materially destructive. In the state of Washington where the adequacy of the one-spray method was vigorously defended for several years, and in most other western states, the calyx spray and four cover sprays finally became the accepted recommendation for control; while in parts of Arkansas, New Mexico, Colorado and California, accentuated conditions of infestation have led to the application of from five to ten cover sprays during the summer.

Attention turned more and more to the belief that incorrect timing of cover sprays, rather than deficiencies in applying the calyx spray, was largely responsible for unsatisfactory control. Cordley,[30] who was among the first to take this point of view, expressed the belief that applying a spray "a few days too early or too late may make all the difference between success and failure," and Childs[51] stated that spraying ten or twelve days before egg-hatching would mean "in ordinary seasons of infestation the difference between complete control as against one-half or even less control." Similar beliefs have been expressed by many other students of codling moth control and efforts at the present time are chiefly directed toward improving the effectiveness of spraying through more accurate timing of spray applications.

THOROUGHNESS IN APPLYING SPRAY

While thoroughness in spraying has always been emphasized, it especially has been the subject of experimentation and extensive discussion during the past ten or fifteen years. The agitation regarding thoroughness has brought forth various kinds of spray nozzles and high-pressured spraying machines capable of producing from three hundred to five hundred pounds pressure. Some authorities have contended that the spray gun or some particular nozzle is especially effective in accomplishing thoroughness and controlling the codling moth while other authorities have presented equally convincing evidence to the contrary. The relative merits of the spray rod and the spray gun in applying the calyx spray and cover sprays, and the relative merits of the disc nozzle and bordeaux nozzle in the calyx application, are points on which there is much confusion of opinion and experimental data. During the past few years the majority of recommendations have called for high pressure, two hundred and fifty pounds or more, but a good many experiments have been reported which indicate that equally good or better control may be obtained by applying spray at pressures of two hundred pounds or less.

The subject of thoroughness involves the question of what kind of spray coverage is most effective. Forbes[6] seems to have been the first to suggest the mist type of coverage. It appears that most authorities have believed this coverage to be the most effective of any. Spray spreaders were used in codling moth control by Klee[7] as early as 1887. They have been experimented with since then by many investigators but only during the past few years has the film type of coverage, obtained by the use of spreaders, come into prominence.

KIND AND QUANTITY OF POISON

Paris green was the principal poison used in spraying for codling moth during the period from 1880 to about 1905. Lead arsenate was employed as early as 1895[42] but the first experiments of consequence were made in 1902. It has been used almost exclusively during the past fifteen years.

The matter of poison concentration in sprays has received comparatively little attention. The prevailing belief among authorities has been that the effectiveness of the spray depends not so much upon the quantity of poison on the apple as upon the thoroughness with which the apple is covered with the poison. Melander,[48] in reviewing methods of codling moth control in the Pacific Northwest, remarked that "experimental tests have shown that the strength of the spray is immaterial." Weldon[46] collected data from orchards in Colorado in 1909 and found that better control was obtained with lower concentrations. Melander later expressed the belief that if the apple is heavily coated with lead arsenate, the newly hatched larva finds the skin distasteful and rejects it.[50] Lovett[54] and Childs and Lovett[56] inclined toward the opinion that lead arsenate in concentration of two pounds to one hundred gallons of water is eight times the theoretical strength required to control the codling moth. At present there seems to be general agreement on the recommendation which has prevailed for several years, that nothing is gained by using lead arsenate in greater concentration than two pounds to one hundred gallons of water.

PRESENT STATUS OF SPRAYING

Notwithstanding that great advances have been made in the perfecting of arsenical compounds and spraying machinery, and in knowledge of the life history of the codling moth, control of the pest in some parts of the country seems to be no more satisfactory now than it was ten or twenty years ago. A careful perusal of horticultural and entomological literature in the Pacific Northwest discloses substantial evidence that the percentage of apples damaged by the codling moth in that region during the period from 1919 to 1924 inclusive, was fully as great as during any previous equal period. In 1919 Melander, who had been continuously interested in codling moth control in the State of Washington for upwards of twenty years, stated that "undoubtedly the codling moth has been increasing in destructiveness during the last few years."[52] The Grand Valley district of western Colorado affords a striking example of the failure of spray-methods to keep pace with the increasing codling moth hazard.[57]

The spray treatment is not nearly as dependable as is desired. One year it may prove very effective in a given district but another year it may give unsatisfactory control, or, in one orchard it may be effective and in an adjoining orchard very ineffective. Such variations in results have been attributed principally to differences in the timing and thoroughness of spray applications. In general, however, there is a wide gap between the degree of control attainable under orchard conditions and the one hundred per cent control that almost all spray authorities have assumed lead arsenate capable of giving. It seems quite evident that there are important factors bearing on the efficacy of lead arsenate in controlling the codling moth, which have not yet been ascertained.

STUDIES WITH FRESHLY HATCHED CODLING MOTH LARVAE

Methods and Technique

A special effort was made to conduct the different experiments under uniform conditions and, so far as possible, to eliminate such variable factors as might contribute to non-comparability and error in the results. After some experience it was found necessary to outline in detail each separate manipulation. Even with very careful attention to methods and technique, the variation in the results of repeated tests was much greater than was expected or desired. Some of the more important points that were followed in performing the various experiments are briefly outlined in following paragraphs:

Securing freshly hatched larvae.—By placing burlap bands around the trunks of apple trees badly infested with codling moth, thousands of mature larvae were captured. The larvae were placed in fruit jars containing strips of corrugated strawboard in which pupation took place. The moths were allowed to emerge in a cage covered with cheesecloth. From this they were transferred to battery jars. A covering of wet sand about one-half inch thick had been previously placed in the bottom of each jar. Sections of glass on which the moths might rest and oviposit were sometimes placed in the jars. The jars were wrapped in four thicknesses of black cheesecloth and kept in a room where a temperature of approximately 80° F. was automatically maintained. The black cloth over the jars caused the moths to oviposit during the day. After some experience with the moths it was found possible to secure from a few hundred to over a thousand eggs in each jar within a period of three days (fig. 1). At the end of the third day, and sooner in some cases, the moths were released and the wing scales, fecal material and sand washed from the jars by using an abundance of tap water. If the jars were not thoroughly washed out it was found that some larvae swallowed particles of the loose substances. Rooms in which high or low temperatures could be automatically maintained were accessible for hastening or retarding as desired the processes of transformation, egg-laying and incubation.

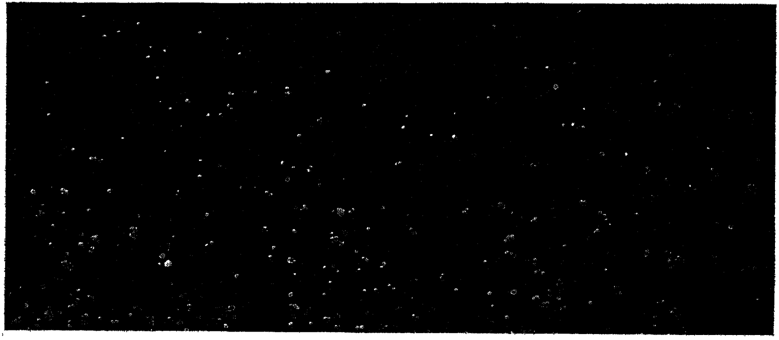
Fig. 1. A section of glass after removal from a battery jar containing moths. Approximately six hundred eggs may be counted on this section.

Preparation of apples.—Only apples from unsprayed trees were used in the studies. In collecting the apples, each was grasped by the thumb and fore finger while the stem was clipped off close to the fruit spur. All handling thereafter was done by holding the stems with forceps. Touching the apples with the hands affected the surfaces in such manner as to influence the spray covering subsequently applied. In the laboratory the calyx lobes were clipped off if they protruded and the calyx cavities filled with shellac. It was found important that the shellac be even with the surrounding surface of the apple because any ridge or protuberance materially aided the larvae in effecting injuries. A piece of cotton thread was tied to the stem of each apple. The transverse and vertical diameters were then measured and recorded.

Technique in spraying apples.—In the work of 1920 and 1921 an atomizer operated by blowing the breath through it was used for applying the spray. It was later discovered, however, that the carbon dioxide of the breath caused a marked increase in the thickness of the film coverage when casein-lime was employed as a spreader; consequently, a rubber bulb type of atomizer was used in the studies subsequent to 1921. The atomizer was kept in constant agitation while applying the spray in order to have the lead arsenate uniformly in suspension. Uniformity of suspension was not satisfactorily maintained in case of the concentrations of eight and sixteen pounds to one hundred gallons.

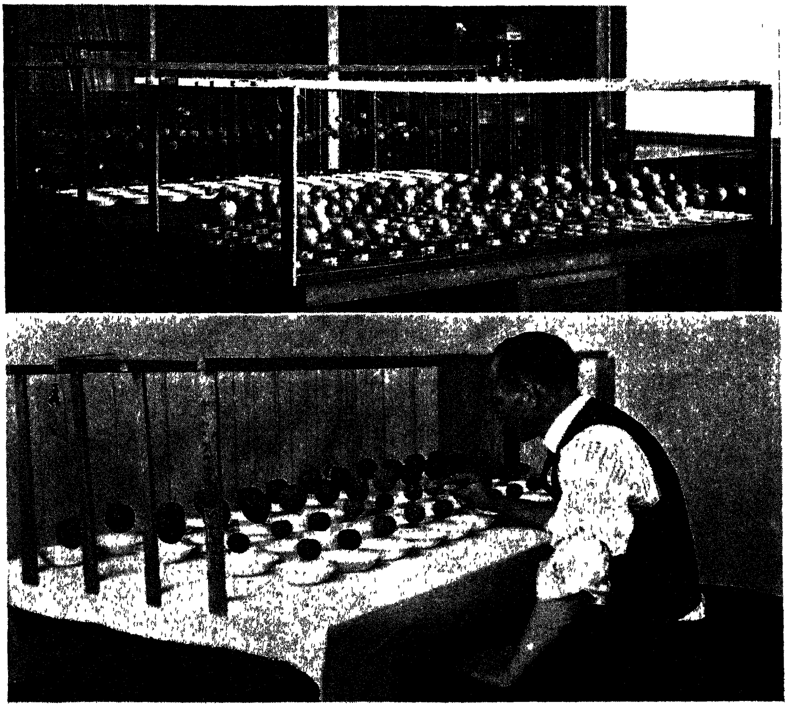

Fig. 2. Upper: several series of apples in the studies of 1924, showing manner in which apples were suspended over vessels of water. Lower: illustration of studies made in the summer of 1923.

In applying the spray, each apple was suspended and given a rotating movement. The entire surface was then sprayed uniformly until the desired type of coverage was effected. Except in the case of the mist coverage, the atomizer was first placed near the base of the stem and the basin about the stem filled with spray. This spray was withdrawn by means of a pipette as soon as spraying of the apple was completed. When casein-lime spreader was used to effect the film coverage, the large drop or drops which formed on the lower surface of the apple were removed by touching that part with blotting paper immediately after spraying. This was done in order to secure more accurate data on the relation of the thickness of the film coverage to protectiveness. After being sprayed the apples were suspended on racks (fig. 2). A vessel of water was placed beneath each apple and in many of the tests a small quantity of tanglefoot was placed on the thread just above the stem. By this means any larvae that fell off of the apples or attempted to crawl up the thread were trapped. Very few were caught in the tanglefoot but many fell off in the water.

Transferring the larvae.—Many factors relating to the transferring of the larvae were found to make for error in the results. There was often much variation in the vigor and size of different larvae, even among those hatching at the same time. Larvae hatching between dawn and about ten o'clock in the morning seemed to be stronger and more likely to produce injuries than those hatching at mid-day or during the afternoon. The first larvae to hatch from a given lot of eggs showed greater vigor than those last to hatch. It also seemed that larvae hatching from eggs whose development had been retarded by being placed in a room with a temperature of about 50° F. were less vigorous than those whose development in the egg had been unchecked. In order to equalize this variation in vitality, the larvae were transferred to the apples in rotation. Five larvae were placed on the first apple, five on the second apple, five on the third, etc., until each apple of a series had five larvae. This was then repeated until a total of twenty-five larvae had been transferred to each apple.

A finely pointed artists' brush, size No. 1, was used for handling the larvae. The brush was kept soft and flexible by moistening it frequently. Considerable care had to be exercised in order to place the larva ventral side down on the apple so that it could make contact with the surface with its legs and spinneret. In case of the spotted coverages an endeavor was made always to place the larvae on the areas between the deposits of poison. Twenty-five larvae to each apple was the standard number used. Special tests were made in which the number ranged from five to two hundred per apple.

Laboratory conditions.—The studies of 1924 were conducted in the northwest room of a building. The apples were exposed to diffused light which entered through large windows on the north and west. The temperature of the room averaged approximately 72° F. but there was considerable variation owing to the fact that the heating plant was undergoing repair. In the series of tests, numbers 15 to 20 inclusive, shown in table 1, the temperature fell to about 60° F. soon after the larvae had been transferred. This probably was responsible for the much lower percentage of injury caused to the apples of these series. The day temperature in Berkeley, where the tests of 1924 were performed, commonly falls below 60° F. in July and August. This also holds true for San Francisco where the 1923 studies were made. On the whole, the laboratory conditions were not satisfactory as regards temperature.

Types of spray coverages.—When a suspension of lead arsenate in water is applied in finely atomized form to the surface of an apple, the liquid particles tend to collect into droplets. Upon evaporation of the water isolated deposits of the compound remain, resulting in what may be termed a spotted spray coverage. By placing in the spray suspension some substance that sufficiently lowers the surface tension, the droplets will coalesce and form a continuous liquid film. Upon evaporation of the water an unbroken film of the compound remains. This has been termed the film spray coverage in this paper. The substance added to the spray for the purpose of producing the film coverage is known as a spreader.

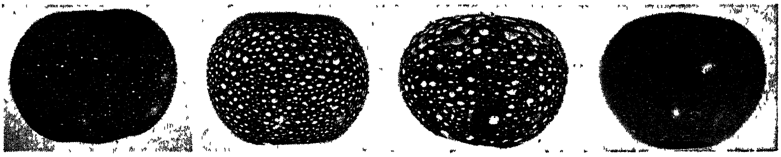

Fig. 3. The four types of spray coverages that may be produced on the surfaces of apples. Left to right: mist coverage, coarse coverage, overspray coverage film coverage.

In the studies of 1920 and 1921 tests were made of two coverages; the film coverage and a coverage that, for the most part, consisted of small spots. The results of the tests were so variable that it was found necessary to differentiate among spotted coverages. In subsequent work three types of spotted coverages were distinguished; the mist coverage, the coarse coverage and the overspray coverage. The mist coverage was produced by exposing momentarily the whole surface of the apple to a finely atomized spray. This resulted in a covering of isolated spots ranging in size up to approximately two millimeters in diameter. The coarse coverage was produced by applying the finely atomized spray continuously until the drops that formed on the surface of the apple were as large as would remain in place. The aim was to stop spraying just before any drop became large enough to run down. The overspray coverage was produced by applying the finely atomized spray in such quantity that large drops collected and ran down more or less irregularly over the apple.

Particular attention is called to the fact that notwithstanding the technical manner in which the spraying was done, there was considerable variation in the character of each type of coverage on different apples. This was especially true of the mist and coarse coverages. Sometimes the spots on the side of an apple were much larger than those on the bottom or top. In some of the tests it was noted that about two-thirds of the surface of an apple was covered fairly evenly with drops of almost maximum size while the remaining surface had scarcely more than a mist coverage. In order to prevent drops from running off, it was necessary to leave some parts improperly covered. Owing to a very slight coating of dust or possibly to some other condition of the surface, larger drops would collect on some apples than on others. On some there was an appreciable tendency for the drops to spread, resulting in spots more or less irregular in outline. This lack of uniformity in coverage was probably responsible for much of the variation in the number of larvae causing injury to apples in different tests.

Materials used.—The lead arsenate used represented two makes: Sherwin-Williams and General Chemical Company. Both were powdered acid lead arsenate ($PbHAsO_4$). The contents of two one-pound commercial cartons were thoroughly mixed together. Analysis showed this to contain 31.2 per cent of arsenic oxide and 0.11 per cent of arsenic oxide in soluble form. The solubility test was made by placing a quantity of the lead arsenate in distilled water at approximately 76° F. for twenty-four hours.

The spreader used for the film coverage consisted of a mixture of casein and calcium hydrate in proportions of twenty-five per cent casein and seventy-five per cent calcium hydrate. This was used at the rate of one pound to one hundred gallons of spray. The casein was a readily soluble form and the calcium hydrate was practically free of carbonates. In all instances distilled water was used and the spray applied within a few minutes after being prepared.

Determination of injury.—Two forms of injury were distinguished: entrances and stings. An entrance corresponds to the term "worm" as commonly used in codling moth writings. It refers to the injury produced by a larva that has burrowed through the skin and into the tissue of the apple without having become poisoned. A sting refers to the injury caused by a larva that has attempted to enter an apple but because of the effects of poisoning or for other causes ceased burrowing after making a slight pit in the skin or, at most, a small excavation in the tissue of the apple.

It was found that in entering the apple the larva generally makes a small excavation under the skin and sometimes spends two or three days apparently feeding in the excavation before starting to burrow toward the center of the apple. The apples, therefore, were left suspended for six days after the larvae had been transferred to them. On the sixth day each apple was submerged in hot nitric acid to dissolve off the arsenic. This treatment removed all frass and excremental material and, in addition, caused a pronounced discoloration at each entrance and sting, which greatly simplified the task of determining injuries (fig. 4).

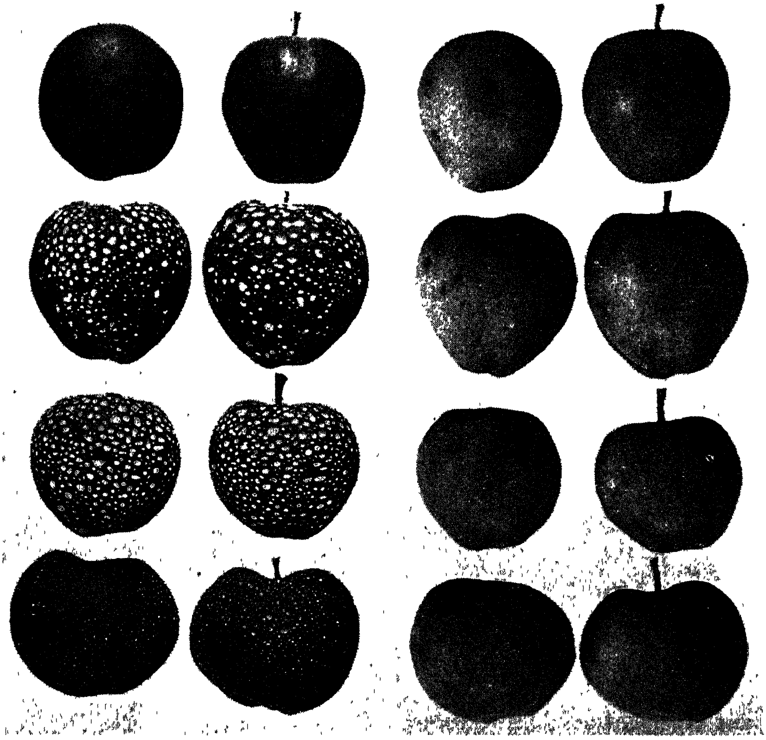

Fig. 4. Left: apples cut into halves showing condition of spray coverages just before submerging in hot nitric acid solution. Right: the same apples after the arsenic had been dissolved off.

An incision approximately one-fourth inch deep was made under each injury. Any burrow that extended deeper than this was recorded as an entrance. Injuries of lesser extent were considered stings unless the larvae were found in such stages of development that they doubtless had been alive at the time of dissolving off the arsenic.

It should be especially noted here that a fairly large percentage of the stings recorded in following tables were so slight that they would have escaped notice on the surface of a growing apple. Considerable care had to be exercised in collecting apples from trees in order to avoid the use of any having skin blemishes which would be confused with sting injuries later on in the experiments. Such irregularities would also materially aid the larvae in producing injury.

Determination of arsenic.—A simplified method was employed in determining the amount of arsenic on the apples. A solution of nitric acid was made up, consisting of fifteen parts of arsenic-free, concentrated nitric acid and eighty-five parts of distilled water. A beaker containing a sufficient quantity of this solution in which to submerge the apple was placed over a Bunsen flame and the solution brought to the boiling point. The apple was placed in the beaker and rotated with a glass rod for about one-half minute while the solution boiled strongly. It then was impaled on the glass rod, lifted from the solution and the surface washed with a stream of hot, dilute nitric acid.

Determinations were made of the amount of arsenic on four apples at a time, each group of four apples being from successive series of tests. Sufficient arsenic was present on any four apples to enable determination by titration. The amount of arsenious oxide, expressed in micromilligrams,* was divided by the total area of each four apples. The area was expressed in square centimeters. This gave the average number of micromilligrams of arsenious oxide per square centimeter of apple surface. The surface of each apple was computed by using the average of the transverse and vertical diameters.

LARVAE ON APPLES SPRAYED WITH LEAD ARSENATE

The studies reported in this paper comprise records of over fifteen thousand freshly hatched codling moth larvae. Of this number over twelve thousand were placed directly onto apples, twenty-five being placed onto each of four hundred eighty-three apples. A number of experiments were made with apples hanging naturally on trees in the orchard.

Studies Made in the Laboratory

The results of the most extensive single study are given in detail in table 1. This study included tests of seventeen different combinations of spray coverages and lead arsenate concentrations. The table gives the number of larvae effecting entrances, the number making stings and the total number causing injury on each apple. The data are summarized in table 4.

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* One micromilligram equals one-thousandth of a milligram.

Observations indicated that only rarely would a larva make more than one injury. The behavior of twenty-two larvae on unsprayed apples was studied in detail from the time of hatching until entrance was made. In no case was it observed that a larva quit an attempted entrance and later started another. In this paper, therefore, it has been considered that the number of injuries corresponds with the number of larvae making them; ten injuries, for example, represent the activity of ten different larvae.

The following varieties of apples were used in the tests shown in table 1:

White Winter Pearmain; series 1, 2, 15, 16.
Stayman Winesap; series, 3, 4, 8, 10, 13, 14, 17, 18, 19, 20.
Yellow Bellflower; series 5, 6, 7, 9, 11, 12.

The Yellow Bellflower seemed to be somewhat more susceptible to injury than the other varieties.

The studies were made during the latter half of July and the first half of August. The apples were fairly large in size.

STUDIES MADE IN THE ORCHARD

The suggestion was made that perhaps a smaller percentage of injury would have occurred if the tests had been made in an orchard environment. In order to secure information on this point, some experiments were performed with apples hanging in their natural positions on the trees.

These tests were made the last week of July with Yellow Bellflower apples. The calyx cavity of each apple was filled with shellac and each was sprayed as nearly as possible in the same manner as was done in the laboratory tests. A band of cotton was tied about the branch on either side of each apple in order to prevent larvae hatching elsewhere on the tree from reaching the test apples. Larvae that had hatched in the laboratory were taken to the orchard and transferred to the apples between nine o'clock and noon of the same day. The results of the experiments are shown in table 2. The temperature in the orchard was approximately 82° F. at noon on the day that the larvae were transferred. The light was much more intense than that in the laboratory tests although the apples selected were on the shaded sides of the trees. The more intense light and the higher temperature tended to accelerate the activity of the larvae, with the result that a larger percentage dropped from the apples.

TABLE 2

RESULTS OF PLACING TWENTY-FIVE FRESHLY HATCHED CODLING MOTH LARVAE EACH ON APPLES HANGING NATURALLY ON TREES

Series of apples	Lead arsenate, 2 lb. to 100 gal.								
	Mist coverage			Coarse coverage			Film coverage		
	Entrances	Stings	Total	Entrances	Stings	Total	Entrances	Stings	Total
1	18	2	20	8	3	11	6	4	10
2	12	0	12	6	4	10	7	2	9
3	17	0	17	11	3	14	8	4	12
4	....	....	(1)	5	1	6	10	2	12
Total.........	47	2	49	30	11	41	31	12	43
Per cent..........	62 7	1.7	66.7	30 0	11.0	41.0	31.0	12.0	43.0

Series of apples	Lead arsenate, 4 lb. to 100 gal.						Check		
	Coarse coverage			Film coverage			Unsprayed		
	Entrances	Stings	Total	Entrances	Stings	Total	Entrances	Stings	Total
1	4	6	10	4	6	10	22	0	22
2	3	2	5	7	2	9	18	0	18
3	4	3	7	4	6	10	16	0	16
4	....	....	(1)	....	....	(1)	20	0	20
Total............	11	11	22	15	14	29	76	0	76
Per cent............	14.7	14 7	29 3	20.0	18.7	38 7	76.0	0.0	76.0

(1) Tests omitted because of accidents or insufficient larvae.

The following comparison of the results of the laboratory and the orchard tests indicates that the spray may be slightly more effective under orchard conditions, although the differences shown easily fall within the range of experimental error.

TABLE 3

A COMPARISON OF THE PERCENTAGES OF LARVAE CAUSING INJURY IN LABORATORY AND ORCHARD TESTS

	Laboratory tests			Orchard tests		
	Entrances, per cent	Stings, per cent	Total injury, per cent	Entrances, per cent	Stings, per cents	Total injury, per cent
Lead arsenate, 2 lb. to 100 gal.:						
Mist coverage..................	68.4	6.6	75.0	62.7	1.7	66.7
Coarse coverage............	31.2	12.6	43.8	30 0	11.0	41.0
Film coverage................	33.0	13.2	46.2	31 0	12.0	43.0
Lead arsenate, 4 lb. to 100 gal.:						
Mist coverage..................	54.0	11.2	65.2	....	....	....
Coarse coverage............	24.0	19.2	43.2	14.7	14.7	29.3
Film coverage................	22.0	19.0	41.0	20.0	18.7	38.7

VARIATION IN NUMBER OF INJURIES PER APPLE

Examination of table 1 brings out the fact that there was a wide variation in the results of the tests. The number of entrances among the unsprayed apples varied from sixteen to twenty-four, while with some of the spray combinations the number ranged from eight to twenty-four among apples having the same treatment. It is believed that an important cause for this variation was that too many apples were run at a time. The twenty-two apples of a series required five hundred fifty larvae. Sometimes the larvae hatched rapidly and they could not be properly transferred, while at other times an insufficient number were available to finish a series and it was necessary to complete the transfer on the following day. The range in number of injuries per apple was much less in other studies in which fewer apples were used in a series. Other causes for variations have been mentioned under the topic, Methods and Technique.

RESULTS OF SPECIAL INTEREST

Four facts that are quite contrary to what might have been expected are shown in tables 1 and 2. First, the lead arsenate was comparatively ineffective in protecting the apples. The general belief has been that apples so thoroughly sprayed would be only slightly injured if at all, whereas, with the standard concentration of lead arsenate at two pounds to one hundred gallons over thirty per cent of the larvae entered unharmed and over forty per cent either entered or made stings. Second, the mist coverage was very much less effective than the coverages of large spots, whereas, there has been a widely accepted opinion among spray authorities that in orchard spraying the mist coverage is the most effective. Third, the film coverage gave scarcely any better protection, except with the higher concentrations, than the spotted coverages, whereas, it might have been expected on theoretical grounds that the film coverage would give much greater protection. Fourth, increasing the concentration of the spray resulted in decidedly decreasing the percentage of larvae that succeeded in effecting entrances, whereas, it has been generally believed in orchard spraying that very little if anything is gained by using lead arsenate in greater proportion than two pounds to one hundred gallons of water.

PROTECTIVENESS OF DIFFERENT COVERAGES

A summary of the laboratory tests with spray concentrations of two, four and eight pounds of lead arsenate to one hundred gallons of water, gives the following percentages of injury:

	Entrances, per cent	Stings, per cent	Total injury, per cent
Mist coverage	57.4	10.0	67.4
Coarse coverage	23.4	18.0	41.4
Overspray coverage	24.0	17.6	41.6
Film coverage	21.7	18.1	39.8

This is shown graphically in figure 5.

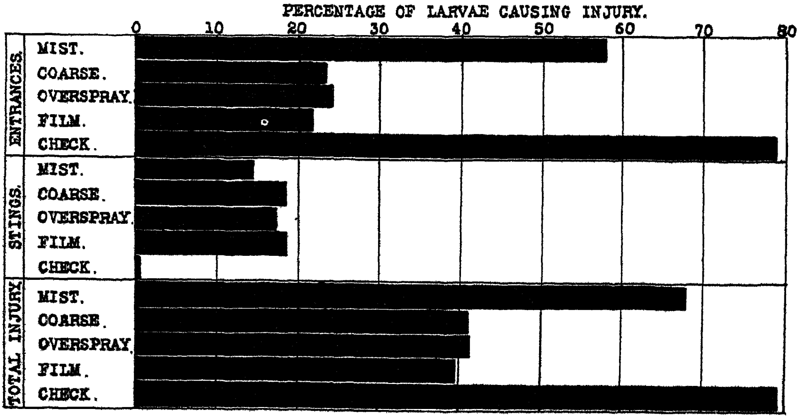

Fig. 5. Graphs based on the data of table 1, indicating the protectiveness of different spray coverages.*

The protectiveness of different coverages is shown in detail in table 4 and by the curves in figure 10. It will be noted that the coarse, overspray and film coverages were about equal in protectiveness up to the four-pound concentration. Above this concentration the film coverage gave greater protection. In all instances the mist coverage gave the poorest protection.

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* The graphs represent the average percentage of larvae causing injury to apples sprayed with concentrations of two, four and eight pounds to one hundred gallons. These were the only concentrations in which tests were made with all four types of coverages. The mist and overspray coverages were omitted in the one-half-pound concentration and the overspray coverage omitted in the sixteen-pound concentration.

TABLE 4

SUMMARY OF THE DATA INCLUDED IN TABLE 1

Coverage and concentration	Entrances, per cent	Stings, per cent	Total injury, per cent
Lead arsenate, ½ lb. to 100 gal.:			
Coarse coverage	57.6	7.2	64.8
Film coverage	55.6	5.8	61.4
Lead arsenate, 2 lb. to 100 gal.:			
Mist coverage	68.4	6.6	75.0
Coarse coverage	31.2	12.6	43.8
Overspray coverage	34.6	14.4	48.9
Film coverage	33.0	13.2	46.2
Lead arsenate, 4 lb. to 100 gal.:			
Mist coverage	54 0	11 2	65.2
Coarse coverage	24.0	19.2	43.2
Overspray coverage	23.3	19.8	43.0
Film coverage	22.0	19.0	41.0
Lead arsenate, 8 lb. to 100 gal.:			
Mist coverage	49.8	12.2	62.0
Coarse coverage	15.6	21.8	37.4
Overspray coverage	15.6	18.6	34 2
Film coverage	10.2	22.2	32.4
Lead arsenate, 16 lb. to 100 gal.:			
Mist coverage	29.2	16.4	45 6
Coarse coverage	9.4	19.0	28.4
Film coverage	2 3	15.8	18.0
Check, unsprayed	79.0	.4	79.4

In the course of the experiments special attention was given to the behavior of the larvae, with a view to finding explanations for the results obtained.

Factors relating to the protectiveness of the mist coverage.—It has long been recognized that some larvae succeed in effecting entrances into sprayed apples by burrowing through the areas between deposits of lead arsenate. Presumably, the larger the deposits, the larger the unprotected areas between deposits and the more room for larvae to enter unharmed by the poison. Owing to this conception the idea has become widely accepted among authorities on spraying that, to be most effective, spray should be applied in such a manner as to cover the apples of a tree with fine particles of mist. The assumption apparently has been that the resultant small deposits of poison would be so close together that there would not be room for larvae to enter between them; that is, the mist coverage on apples has been regarded as practically tantamount to having them covered by an unbroken film. As shown by these studies, this assumption is seriously in error. When the deposits of lead arsenate were from one-half millimeter to two millimeters in diameter, the latter representing an area about the size of the head of an ordinary pin, there was ample space for larvae to enter between the deposits. This is well illustrated by the photographs in figure 6. The photograph on the left shows an apple natural size, having a fairly typical mist coverage of lead arsenate. A freshly hatched codling moth larva was placed on the apple and observed until it started to burrow through the skin. A common pin was then inserted in the side of the apple close to the larva. The microphotograph was then taken. The head of the pin measured slightly less than two millimeters in diameter.

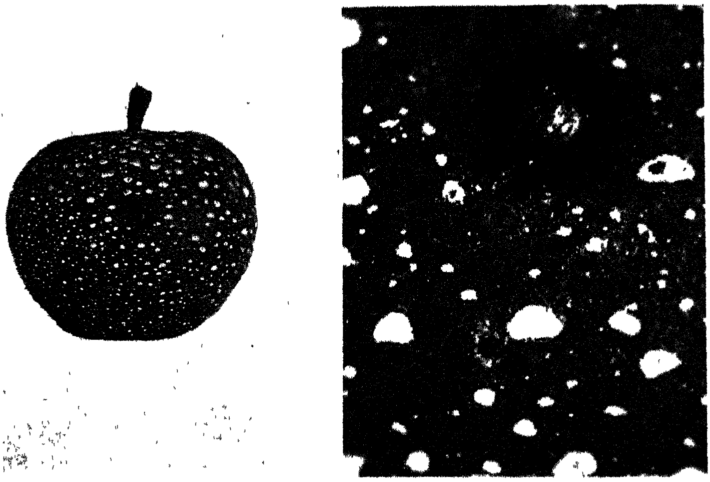

Fig. 6. Left: an apple having a mist coverage of lead arsenate, with a pin inserted in the side. Right: microphotograph of the same apple, showing a freshly hatched codling moth larva in the act of burrowing into the apple.

Writers on codling moth control, wishing to emphasize the necessity of having apples thoroughly covered with spray, have sometimes stated that the larva makes a hole about the size of a pin head on entering the apple. The head of an ordinary pin, however, is more than twenty-five times the area of the entrance hole of a newly hatched larva. The question may be raised whether the larva does not enlarge the hole after entering the apple. Such behavior was not observed in any of these studies, neither were evidences of it found in examining apples in the orchard.

Where the deposits of lead arsenate were very minute, the larvae appeared to burrow through them or to dig them away without being affected by the poison. This was especially the case with the lower concentrations of spray.

Another reason for the lower protectiveness of the mist coverage appeared to be that the small deposits were so nearly even with the surface of the apple that they did not stimulate the thigmotactic proclivities of the larvae, as did the large deposits of the coarse and overspray coverages.

Factors relating to the protectiveness of the coarse coverage.—The largest deposits of the coarse coverage were approximately eight millimeters in diameter. Notwithstanding the numerous large interspaces which appeared to be relatively unprotected, less than one-half as many larvae entered the apples with coarse coverage as entered those with the mist coverage. There seemed to be four reasons why the coarse coverage gave so much better protection than theoretically might have been expected. First, the larvae exhibited a tendency to rest upon and examine the deposits of lead arsenate and it seemed probable that some larvae became poisoned incidentally in doing this. Second, the relatively thick edges of the large deposits stimulated the thigmotactic reactions of the larvae, which resulted in many of them attempting to dig through the edges and thus becoming poisoned. Third, although

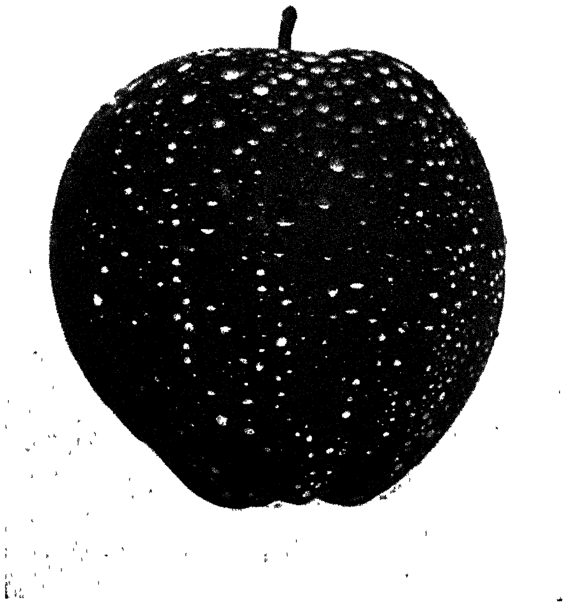

Fig. 7. An overspray coverage of lead arsenate in concentration of two pounds to one hundred gallons of water. The arrow points to an entrance made through the upper part of a deposit.

there appeared to be relatively large unprotected areas between the larger deposits, close examination revealed many small deposits, resembling those of a mist coverage, on these areas. The small deposits were doubtless of some influence in preventing entrances. Fourth, it seemed evident that the apples with the coarse coverage had more surface actually covered with poison than the apples with the mist coverage.

In case of the coarse coverage of one-half pound to one hundred gallons, many entrances were made directly through deposits of poison. With the concentration of two pounds to one hundred gallons, entrances were sometimes made through the thin upper parts of the large deposits. An example of this is shown in figure 7.

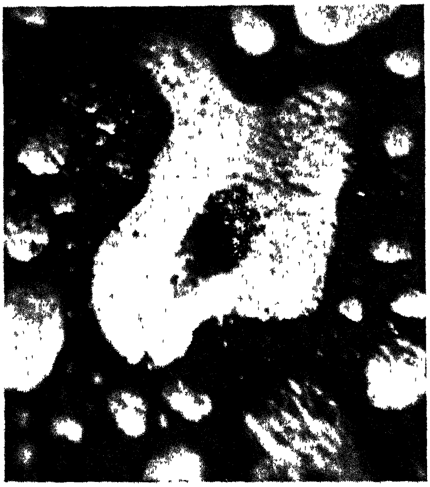

Fig. 8. Microphotograph showing where a larva had burrowed through a heavy deposit of lead arsenate.

Two or three instances were observed where larvae had burrowed directly through heavy deposits of lead arsenate. An example of this is shown in the microphotograph in figure 8. The fresh castings which were being thrown out when the photograph was taken was evidence that the larva was alive. After taking the picture the burrow was opened. The larva had made a typical excavation under the skin. It appeared to be unaffected by the poison but no special observations were made to determine this point with certainty. The probable explanation of such occurrences is discussed near the close of this paper.

Factors relating to the protectiveness of the overspray coverage.—The distribution of the lead arsenate in case of the overspray coverage was much more irregular than that of the coarse coverage. Wherever a large drop of spray ran down just before spraying was stopped, the path of the drop presented a relatively large area which, on casual observation, appeared to bear very little poison. It should be mentioned that special care was taken not to cause any drop to run down after spraying was completed. In general, the oversprayed apples appeared to be less effectively covered than those of the coarse coverage yet the protectiveness of the two coverages was about the same. This fact seemed to be due in part, at least, to three factors: first, it was noticed that some deposits of the overspray coverage were larger and thicker than any of the coarse coverage; second, as soon as a drop ran down other drops immediately began forming in its path; third, it is possible that a thin film of poison may have adhered to the apple skin as the drops ran down.

TABLE 5

RESULTS OF TESTS WITH APPLES OVERSPRAYED, AND WITH APPLES HEAVILY OVERSPRAYED AND THEN LIGHTLY SHAKEN

Series of apples	Lead arsenate, 2 lb. to 100 gal.							
	Typical overspray coverage				Apples heavily oversprayed and lightly shaken			
	Entrances	Stings	Total	mmg. As_2O_3 per sq. cm.	Entrances	Stings	Total	mmg. As_2O_3 per sq. cm.
1	6	4	10		11	3	14	
2	7	7	14		14	3	17	
3	6	4	10		8	5	13	
4	8	4	12		10	6	16	
Total number	27	19	46		43	17	60	
Per cent	27.0	19.0	46.0		43.0	17.0	60.0	
Average				8.63				4.20

The third factor has been a matter of considerable speculation. Some persons have believed that an oversprayed apple is covered by a very thin film as a result of numerous drops running down and it has been supposed that such a film would be effective in preventing injury by codling moth larvae. In order to secure information on this, four apples were heavily oversprayed by applying twenty cubic centimeters of spray to each. Immediately after spraying, the apples were lightly shaken in order to cause the larger drops to run off. Another four apples having a typical overspray coverage were used as checks. Twenty-five freshly hatched larvae were then placed on each of the apples. Finally, determinations were made of the arsenic and computations made of the average number of micromilligrams of arsenious oxide per square centimeter of surface for each group of apples. The results of the test are shown in table 5. The data indicate that if a film of lead arsenate formed on the paths of the drops which ran off, it was of slight effect in protecting the apples. About one-half as much arsenic occurred on the apples heavily sprayed and shaken as on those typically oversprayed.

The coverage resulting from overspraying and shaking probably approximates that resulting from overspraying apple trees in the orchard when wind is blowing. The experiment indicates that almost half the value of the spray may be lost under such a condition.

Factors relating to the protectiveness of the film coverage.—So much emphasis has been placed upon "lack of thoroughness" in applying spray as a leading cause for failure to control the codling moth that a great many persons have come to believe that complete coverage would result in complete protection. When no other explanation for a "wormy" crop of apples has seemed plausible, the sprays being properly timed and the applications being made with exceptional thoroughness, it has been supposed that the poor control was due to the larvae entering between deposits of poison. Spreaders and the film type of coverage were brought into prominence on the theory that if the deposits of lead arsenate could be eliminated and the apples covered with a complete film, much better protection would result.

Examination of tables 1 and 2 show clearly that other factors than completeness of coverage are involved. With the concentrations of one-half, two and four pounds to one hundred gallons, about as many larvae caused injury through the film coverage as through the coarse and overspray coverages. The film coverage of eight and sixteen pounds to one hundred gallons, however, resulted in fewer entrances than the spotted coverages of the same concentrations.

The most important factor in the protectiveness of the film coverage is the thickness of the film. The newly hatched larva digs or burrows through the apple skin; it does not literally eat through. Bits of skin are cut off with the mandibles and cast aside. Very little, and sometimes none at all, is swallowed (see page 446). If the film of lead arsenate is thin, as was the case with the lower concentrations, many larvae will not swallow any poison or not enough to kill them. The thicker the coating of poison, the greater the probability of the larvae obtaining lethal doses of arsenic in digging through the skin. Further evidence on this matter is given in table 8 and figures 12, 13 and 18, and the discussion relating to these.

Two other reasons may be mentioned why the film coverage did not give materially better protection than the spotted coverages: first, the lead arsenate of the film adhered firmly to the surfaces of the apples and this minimized the possibility of the larvae gathering up particles of poison while crawling over the apples; second, the film coverage presented no irregularities which would tend to stimulate the larvae to bite against the poison as apparently occurred in case of the spotted coverages.

APPLES WITH TWO APPLICATIONS OF SPRAY

As a possible explanation of the relative ineffectiveness of the lead arsenate in protecting the apples in the foregoing tests, the suggestion was made that perhaps the spotted coverages would have given better results if two applications of spray had been applied. It was thought that the drops of the second application might form on the uncovered spaces left by the first application. In order to secure information on this point, several tests were made in which apples were sprayed twice, the second application being applied after the first had dried. The calyx cavities were filled with shellac and the apples suspended as in previous tests. Larvae were then place on the apples. The results of these tests are given in table 6. The two applications of spray at two pounds to one hundred gallons gave about the same protection as the one application of spray at four pounds to one hundred gallons shown

TABLE 6

RESULTS OF PLACING TWENTY-FIVE LARVAE EACH ON APPLES RECEIVING TWO APPLICATIONS OF SPRAY. (For the purpose of comparison, results of single spray applications, taken from table 1, are also included.)

Series of apples	Lead arsenate, 2 lb. to 100 gal.											
	Mist coverage			Coarse coverage			Overspray coverage			Film coverage		
	Entrances	Stings	Total	Entrances	Stings	Total	Entrances	Stings	Total	Entrances	Stings	Total
1	16	2	18	4	7	11	2	6	8	4	6	10
2	12	3	15	4	6	10	4	5	9	5	6	11
3	14	1	15	8	2	10	3	5	8	5	4	9
4	10	3	13	6	2	8	8	2	10	5	5	10
Total number	52	9	61	22	17	39	17	18	35	19	21	40
Per cent	52 0	9.0	61.0	22.0	17.0	39.0	17.0	18.0	35.0	19.0	21.0	40.0
Percentage of larvae causing injury in tests of one application. (Table 1.) Lead arsenate:												
2 lb. to 100 gal	68.4	6.6	75.0	31.2	12.6	43.8	34.4	13.8	46.2	33.0	13.2	46.2
4 lb. to 100 gal	54.0	11 2	65.2	24.0	19.2	43.2	23.3	19.8	43.0	22.0	19.0	41.0

in table 1. While applying the spray it was noticed that the drops of the second application invariably formed on the deposits of the first application. Consequently, the interspaces resulting from the two applications were about as large as those of the single application (fig. 9). It appeared that the principal effect of the second spray was that of doubling the thickness of the deposits of the first. Observations in orchard spraying revealed the same tendency of the drops of the second and third applications to collect on the deposits of the first.

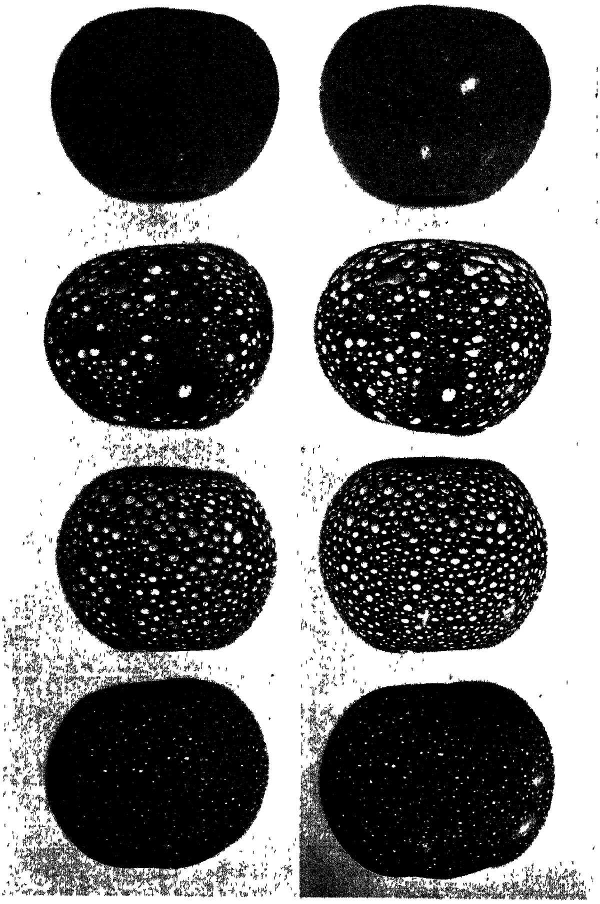

Fig. 9. Left: apples with one application of spray. Right: the same apples after a second application of spray had been applied.

RATIO OF ENTRANCES TO STINGS

It will be observed in all the tests that the percentage of entrances varied inversely with the percentage of stings, except in the concentration of sixteen pounds to one hundred gallons in which there occurred a reduction in both entrances and stings. This tends to confirm the belief advanced by Melander[53] that the ratio of entrances to stings affords the best basis for judging the efficiency of orchard spraying. A high proportion of stings in relation to entrances indicates a high percentage of larvae killed.

QUANTITY OF LEAD ARSENATE IN RELATION TO PROTECTION

Concentration of spray in relation to protection.—As previously mentioned there has been a decided disposition on the part of spray authorities to regard the amount of poison on the apple as a matter of relatively minor importance in codling moth control. Analyses that have been reported have suggested that scarcely more than a trace of lead arsenate over the surface is sufficient to prevent entrances. The conception apparently has been that if one apple was sprayed all over with a concentration of two pounds to one hundred gallons and another sprayed in the same manner with a concentration of four pounds to one hundred gallons, the uncovered interspaces on the one would be just as large and as numerous as on the other. No larva, presumably, would be able to go through a deposit of the lower concentration and, therefore, nothing could be gained by using a higher concentration.

In these studies it was found that increasing the concentration of lead arsenate resulted in all cases in decreasing the percentage of entrances and, to a less extent, in reducing the percentage of total injury. This is graphically illustrated in figure 10. It will be observed that the curve representing the entrances made through the film coverage is nearly a straight line, indicating a fairly constant ratio between concentrations and entrances. The curves of the mist, coarse and overspray coverages are less regular than the curve of the film coverage. It appeared that doubling the concentration in case of the film coverage resulted in uniformly doubling the thickness of the film, thereby uniformly decreasing the percentage of larvae that succeeded in digging through; while doubling the concentration of the spotted coverages resulted in doubling the thickness of the deposits without very materially decreasing the size of the interspaces (fig. 12).

In table 7 is given a summary of the data of table 1, including the average percentage of injury for the four types of spray coverages. The average percentage of injury in relation to concentration is shown

TABLE 7

SUMMARY OF DATA FROM TABLE 1, SHOWING THE RELATION OF LEAD ARSENATE CONCENTRATION TO PERCENTAGE OF LARVAE CAUSING INJURY

Type of coverage	Lead arsenate, 2 lb. to 100 gal.			Lead arsenate, 4 lb. to 100 gal.			Lead arsenate, 8 lb. to 100 gal.			Lead arsenate, 16 lb. to 100 gal.		
	Entrances	Stings	Total	Entrances	Stings	Total	Entrances	Stings	Total	Entrances	Stings	Total
Mist	68.4	6.6	75 0	54 0	11.2	65.2	49.8	12 2	62.0	29 2	16 4	45.6
Coarse	31.2	12.6	43.8	24 0	19.2	43.2	15 6	21.8	37.4	9.4	19.0	28.4
Overspray	34 4	13.8	48.9	23 3	19.8	43.0	15.6	18.6	34.2	....	....	....
Film	33.0	13.2	46.2	22.0	19.0	41.0	10.2	22.2	32.4	2.3	15.8	18.0
Average	42.2	11.6	53.8	31.2	17.2	48.4	22 8	18 7	41.5	11.2	17.3	28 5

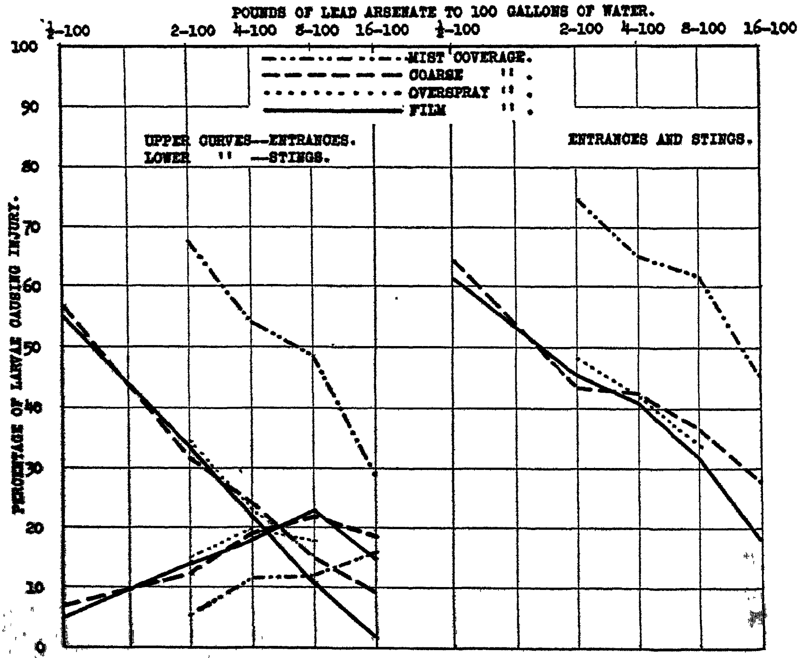

Fig. 10. Curves based on data of table 1, showing relation of lead arsenate concentration to the percentage of larvae causing injury.

by the curves in figure 11. It will be noted by the averages that doubling the amount of lead arsenate resulted in each case in reducing the number of entrances by approximately eleven per cent. Increasing from two pounds to four pounds reduced the total injury 5.4 per cent; increasing from four to eight pounds reduced the total injury 6.9 per cent; increasing from eight to sixteen pounds reduced the total injury 13.0 per cent.

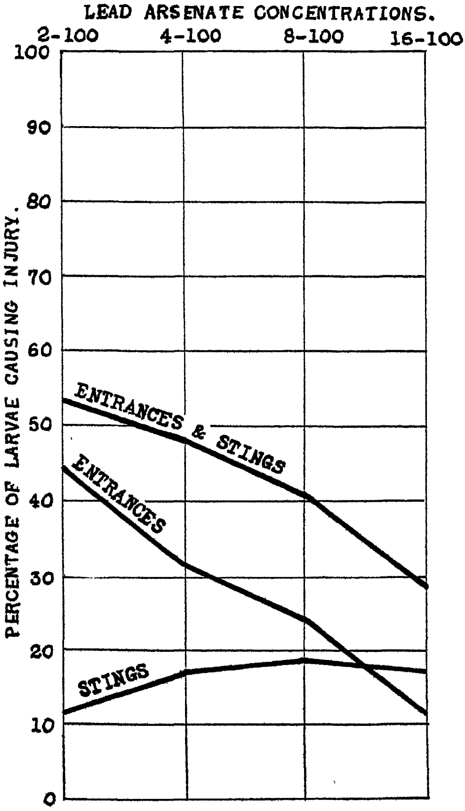

Fig. 11. Curves based on data of table 1, showing average percentage of larvae causing injury through mist, coarse, overspray and film coverages in relation to lead arsenate concentration.

Thickness of lead arsenate deposit in relation to protection.—Determinations were made of the arsenic in all the tests recorded in table 1, and from this was computed the average amount of arsenic per square centimeter of apple surface. The data, together with percentages of injury, are given in condensed form in table 8. In all instances the percentage of entrances varied inversely with the amount of arsenic per square centimeter, while up to the point corresponding to the eight-pound concentration, the percentage of stings varied directly with the amount of arsenic per square centimeter.

Although the film coverage gave slightly better protection than the coarse coverage, the amount of arsenic per square centimeter was about one-third that of the coarse coverage. This indicates that the film coverage is three times as efficient as the coarse coverage. The relative efficiencies of the different coverages are shown by the curves in figure 13. It will be noted that twenty-five micromilligrams of arsenic (As_2O_3) per square centimeter resulted in 2.3 per cent of entrances with the film coverage while seventy-seven and one-half micromilligrams in case of the coarse coverage resulted in 9.4 per cent of entrances. By extending the curves in the first graph, it appears that complete protection against entrances would be secured at about thirty micromilligrams per square centimeter with the film coverage and at about one hundred and fifty micromilligrams with the coarse coverage. Similarly, extending the curves in the second graph, indicates that complete freedom from injury would result at about forty micromilligrams in case of the film coverage and at over two hundred micromilligrams with the coarse coverage. Still further evidence of the greater efficiency of the film coverage is that the percentage of stings began to decrease at approximately fourteen micromilligrams, whereas, decrease in stings in case of the coarse coverage began at approximately thirty-five micromilligrams.

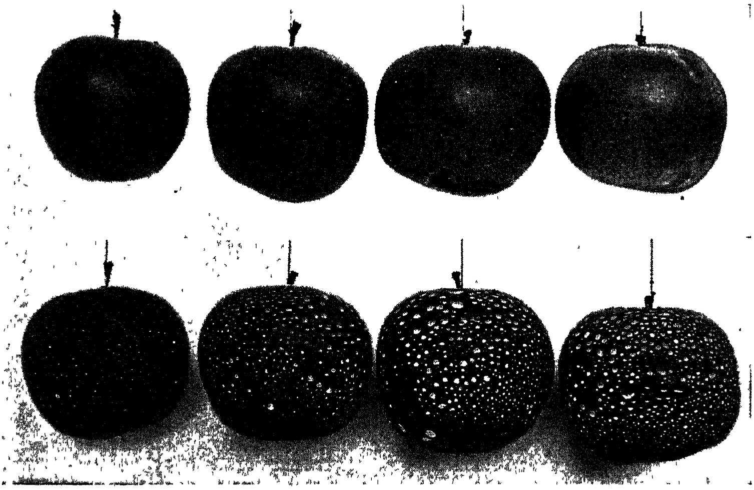

Fig. 12. Apples sprayed with lead arsenate in concentrations of (left to right) one-half, two, four and eight pounds to one hundred gallons. Upper, film coverage; lower, coarse coverage.

TABLE 8

AVERAGE AMOUNT OF ARSENIOUS OXIDE PER SQUARE CENTIMETER OF APPLE SURFACE AND PERCENTAGES OF INJURY FOR THE TESTS SHOWN IN TABLE 1

Lead arsenate concentration	Mist coverage				Coarse coverage			
	mmg. As_2O_3 per sq. cm.	Per cent of injury			mmg. As_2O_3 per sq. cm.	Per cent of injury		
		Entrances	Stings	Total		Entrances	Stings	Total
2 lb. to 100 gal.	2.10	68.4	6.6	75.0	9.78	31.2	12.6	43.8
4 lb. to 100 gal.	3.04	54.0	11.2	65.2	17.45	24.0	19.2	43.2
8 lb. to 100 gal.	4.92	49.8	12.2	62.0	34.70	15.6	21.8	37.4
16 lb. to 100 gal.	13.74	29.2	16.4	45.6	77.51	9.4	19.0	28.4
Average	5.01	53.4	10.9	64.3	34.86	19.9	18.2	38.2

Lead arsenate concentration	Overspray coverage				Film coverage			
	mmg. As_2O_3 per sq. cm.	Per cent of injury			mmg. As_2O_3 per sq. cm.	Per cent of injury		
		Entrances	Stings	Total		Entrances	Stings	Total
2 lb. to 100 gal.	8.20	34.6	14.4	48.9	3.83	33.0	13.2	46.2
4 lb. to 100 gal.	17.17	23.3	19.8	43.0	6.86	22.0	19.0	41.0
8 lb. to 100 gal.	32.83	15.6	18.6	34.2	13.87	10.2	22.2	32.4
16 lb. to 100 gal.	......	......	......	......	24.99	2.3	15.8	18.0
Average	20.65	24.0	17.6	41.6	11.43	17.6	17.6	35.2

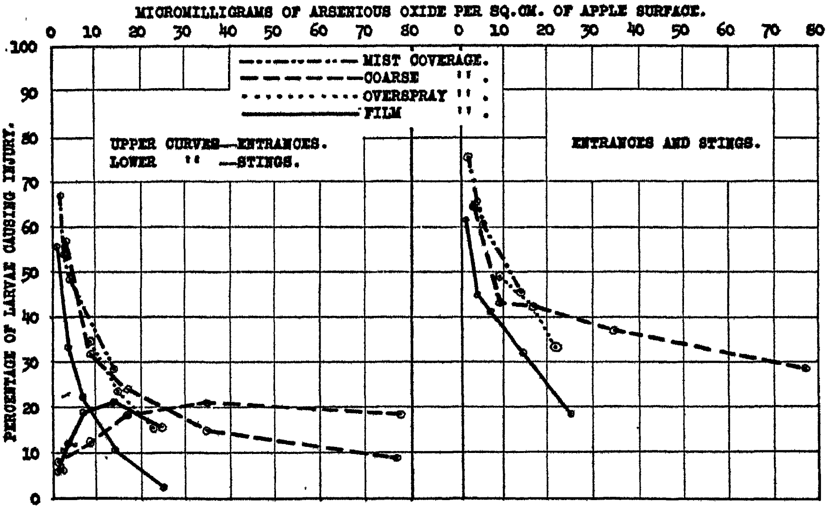

Fig. 13. Curves based on the data of table 8, showing relation of quantity of arsenious oxide per square centimeter of apple surface to percentage of larvae causing injury.

LARVAE ON APPLES DUSTED WITH LEAD ARSENATE

Scores of orchard experiments have been made in various parts of the country to determine the relative merits of applying lead arsenate as a dust and as a spray. The experimental data accumulated have been so erratic that the question of spraying versus dusting is about as unsettled now as it was ten or twenty years ago. The removal of the dust by rain and wind is doubtless an important factor in the efficacy of the dust treatment. It seems almost incredible that in a great many reports of orchard dusting experiments no mention whatsoever has been made of the possible influence wind and rainfall may have had on the results.

Studies Made in the Laboratory

In order to obtain more information on the efficacy of the dusting method of codling moth control, seven series of apples were dusted uniformly with a dry mixture consisting of ninety per cent flowers of sulfur, approximately two-hundred mesh in fineness, and ten per cent lead arsenate. The material was applied with a small hand duster. The calyx cavities of the apples were filled with shellac and the apples suspended as in previous tests. The covering of dust was such as to be decidedly visible to the unaided eye. Certain of the apples were blown against strongly with the breath; others were lightly sprayed with distilled water until drops ran down over the whole surface of each apple; and on others the dust covering was left undisturbed. Twenty-five freshly hatched codling moth larvae were then transferred to each apple. The results of the tests are given in table 9. Blowing against the apples and spraying them greatly reduced the protectiveness of the dust covering. It should be mentioned that the dust was not applied to the apples with force. It was found later that when dust strikes the apple with considerable force, it adheres more firmly than when it falls lightly upon the surface.

Studies Made in the Orchard

Tests on the efficacy of the dust treatment were also made with apples in their natural positions on the trees, similar to the orchard spray tests reported in table 2. The results of these tests, as shown in table 10, agree closely with those made in the laboratory.

The dust coverage when undisturbed was much more effective in comparison with the spray than was expected. Less than one-half as

TABLE 9

RESULTS OF PLACING TWENTY-FIVE LARVAE EACH ONTO APPLES DUSTED WITH LEAD ARSENATE

Series of apples	Dust undisturbed			Apples blown against			Apples lightly sprayed			Check		
	Entrances	Stings	Total	Entrances	Stings	Total	Entrances	Stings	Total	Entrances	Stings	Total
1	2	7	9	7	6	13	11	2	13	18	1	19
2	4	6	10	5	3	8	12	0	12	18	2	20
3	4	3	7	11	1	12	16	0	16	16	0	16
4	2	2	4	13	0	13	15	2	17	21	0	21
5	5	1	6	14	0	14	16	0	16	17	0	17
6	1	3	4	1	5	6	10	3	13	17	1	18
7	5	3	8	2	2	4	12	2	14	17	0	17
Total number	23	25	48	53	17	70	92	9	101	127	4	131
Per cent	13.1	14.3	27.5	30.3	9.7	40.0	52.6	5.2	57.7	72.6	2.3	74.9

many entrances were made through the dust coverage as through the coverage of spray in concentration of two pounds of lead arsenate to one hundred gallons of water. Observations on the behavior of the larvae indicate that the reasons for the greater protectiveness of the dust are: first, the larva is more likely to gather up and swallow particles of poison on the dusted apple than on the sprayed; second, the dust accumulates on the spinneret and on other parts of the head and body, which greatly impedes the activity of the larva. Preparatory to digging into the apple, the larva spins a more or less distinct mat of fibers with which to hold fast while cutting away the skin. The dust

TABLE 10

RESULTS OF PLACING TWENTY-FIVE LARVAE EACH ONTO DUSTED APPLES HANGING NATURALLY ON TREES

Series of apples	Dust undisturbed			Apples blown against			Apples lightly sprayed			Check		
	Entrances	Stings	Total	Entrances	Stings	Total	Entrances	Stings	Total	Entrances	Stings	Total
1	3	6	9	9	4	13	12	3	15	22	0	22
2	3	4	7	11	2	13	14	0	14	18	0	18
3	5	4	9	5	6	11	9	3	12	16	0	16
4	2	2	4	6	4	10	12	2	14	20	0	20
Total number	13	16	29	31	16	47	47	8	55	76	0	76
Per cent	13	16	29	31	16	47	47	8	55	76	0	76

seems especially to frustrate the larva in this operation. If the dust coating adheres firmly to the surface of the apple, however, the larva is not appreciably inconvenienced. Apples which had a heavy firm covering of road dust were entered by larvae just as readily as apples having no dust covering.

LARVAE ON APPLE LEAVES SPRAYED WITH LEAD ARSENATE

During the months of May and June when the apples are small a high percentage of codling moth eggs are laid on the leaves of apple trees. It has been supposed that many larvae become poisoned by feeding on sprayed leaves before finding apples. The following laboratory studies were made to obtain further information on this subject.

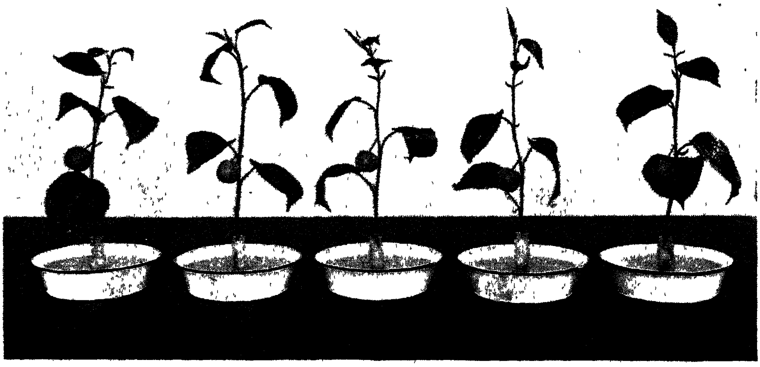

Fig. 14. Cuttings of apple branches ready for tests.

Early in June growing ends of apple branches were cut off and placed in vessels of water, as shown in figure 14. Twenty-five cuttings, eighteen inches long, were used. Care was taken to select cuttings with the same number of leaves and in other respects alike. The upper and under surfaces of the leaves and the bark of certain of the cuttings were sprayed with lead arsenate. An unsprayed apple was fastened to each cutting by diagonally clipping off the apple stem, covering it with glue and then firmly fixing the cut end to the bark with a small insect pin. In other tests the leaves were left unsprayed and sprayed apples were fastened to the bark. The calyx cavities of the apples were filled with shellac. The coarse coverage of lead arsenate spray was used on both leaves and apples. After the spray had dried, twenty-five freshly hatched larvae were placed on the fourth leaf above the apple on each cutting. The larvae had to crawl approximately the same distance on each cutting in order to reach the apples. Three days after transferring the larvae, careful examinations were made of the apples, leaves and stems, and records made of the number of live larvae and of various injuries. The results of the tests are given in table 11.

TABLE 11

SHOWING THE RESULTS OF TRANSFERRING TWENTY-FIVE LARVAE EACH TO CUTTINGS FROM THE GROWING ENDS OF APPLE BRANCHES TO WHICH APPLES WERE ARTIFICIALLY ATTACHED

Treatment	Cutting	Live larvae in apples	Live larvae in stems	Live larvae in leaves	Total live larvae	Stings on apples	Feeding places on stems	Feeding places on leaves	Total feeding places on leaves and stems
Check—unsprayed.	1	15	5	1	21	0	0	1	1
	2	11	3	2	16	0	2	5	7
	3	12	3	5	20	0	2	2	4
	4	9	3	4	16	0	1	3	4
	5	7	2	4	13	0	2	4	6
Total number		54	16	16	86	0	7	15	22
Per cent		43.2	12 8	12.8	68 8	0.0	5.6	12.0	16.0
Leaves sprayed with lead arsenate, 2 lb. to 100 gal.	1	10	2	0	12	0	0	3	3
	2	6	2	2	10	0	3	1	4
	3	6	0	2	8	0	1	3	4
	4	3	2	2	7	0	1	3	4
	5	3	0	0	3	0	1	1	2
Total number		28	6	6	40	0	6	11	17
Per cent		22.4	4.8	4.8	32.0	0.0	4.8	8.0	13.6
Apples sprayed with lead arsenate, 2 lb. to 100 gal.	1	6	1	1	8	2	2	5	7
	2	6	1	2	9	1	2	1	3
	3	5	4	1	10	0	1	3	4
	4	10	3	0	13	1	2	1	3
	5	3	1	4	8	0	2	2	4
Total number		30	10	8	48	4	9	12	21
Per cent		24.0	8.0	6.4	38.4	3 2	7.2	9.6	16 8
Leaves sprayed with lead arsenate, ½ lb. to 100 gal.	1	12	2	2	16	0	0	0	0
	2	7	1	3	11	0	1	3	4
	3	11	1	2	14	0	2	4	6
	4	9	1	0	10	0	1	4	5
	5	6	1	4	11	0	2	1	3
Total number		45	6	11	62	0	6	12	18
Per cent		36.0	4.8	8.8	49.6	0.0	4 8	9.6	14.4
Leaves sprayed with lead arsenate, 4 lb. to 100 gal.	1	7	0	0	7	1	1	2	3
	2	5	0	0	5	0	0	3	3
	3	3	1	0	4	0	0	6	6
	4	2	0	1	3	0	1	1	2
	5	0	0	1	1	0	2	2	4
Total number		17	1	2	20	1	4	14	18
Per cent		13.6	0.8	1.6	16.0	0.8	3.2	11.2	14.4

RELATIVE VALUE OF SPRAY ON LEAVES AND ON APPLES

The relative value of spray on the leaves and bark, and on the apples is best indicated by the number of live larvae found at the end of the third day after transferring. These data are summarized in table 12. A comparison of the concentrations of two pounds to one hundred gallons, shown in columns three and five, indicates that more larvae were killed by the spray on the leaves and bark than by the spray on the apples. The extensiveness of injury to the leaves and stems, however, was very much greater than that which occurs under orchard conditions. Examination, for example, of eight branches on which were 4744 leaves and sixteen apples, on a tree so badly infested with codling moth that there was an average of twelve injuries per apple, revealed only three feeding burrows in leaves and stems. This examination was made on August 22. It is believed, therefore, that only limited importance may be attached to the laboratory tests as regards the relative value of spray on leaves and on fruit.

TABLE 12

SUMMARY OF DATA ON TESTS WITH CUTTINGS FROM GROWING ENDS OF APPLE BRANCHES

	Check unsprayed	Leaves and bark sprayed with lead arsenate			Apples sprayed lead arsenate
		½ lb. to 100 gal.	2 lb. to 100 gal.	4 lb. to 100 gal.	2 lb. to 100 gal.
Live larvae in apples	43 2	36 0	22.4	13.6	24.0
Live larvae in stems	12 8	4.8	4.8	0 8	8 0
Live larvae in leaves	12.8	8.8	4.8	1.6	6.4
Total	68.8	49.6	32 0	16.0	38.4

It is especially important to note that increasing the concentration of the lead arsenate in spraying the cuttings resulted in decidedly increasing the percentage of larvae killed. Two pounds to one hundred gallons resulted in thirty-two per cent live larvae and four pounds to one hundred gallons resulted in sixteen per cent live larvae.

LARVAE ON SECTIONS OF GLASS SPRAYED AND DUSTED WITH LEAD ARSENATE

In order to obtain further data on the relative protectiveness of different types of spray coverages and different lead arsenate concentrations, and also to secure further information on the manner in which codling moth larvae become poisoned, a number of studies were made in which freshly hatched larvae were placed on sections of glass treated with lead arsenate and thence transferred to unsprayed apples. Ordinary window glass was cut into sections three inches by fifteen inches in size. A piece of thread was fastened with sealing wax to the top of each section. Some of the sections were sprayed and some dusted uniformly on both sides. Tests were made of mist, coarse and film coverages with lead arsenate in concentrations of two and eight pounds to one hundred gallons. The dust was a mixture of ninety per cent sulfur and ten per cent lead arsenate, the same as used in the tests with apples. The sections were suspended over saucers of water and freshly hatched larvae placed upon them. In each case the larvae were placed near the middle of the section, half being placed on one side and half on the other. After remaining on the sections for periods

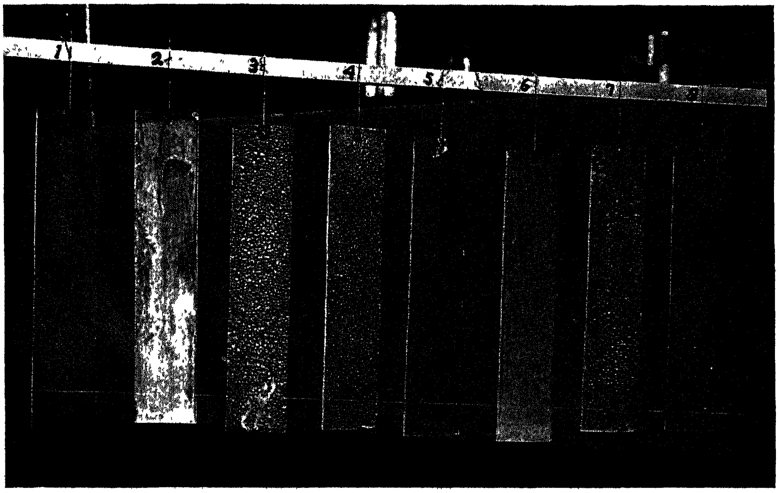

Fig. 15. Sections of glass sprayed and dusted with lead arsenate, on which freshly hatched larvae were placed.

TABLE 13

RESULTS OF TESTS IN WHICH LARVAE WERE PLACED ON SPRAYED AND DUSTED SECTIONS OF GLASS AND LATER TRANSFERRED TO UNTREATED APPLES

	Type of cover-age	Hours larvae remained on sections	Number of larvae placed on sections	Percent-age fall-ing off of sections	Percent-age dying on sections	Number trans-ferred to apples	Number making entrances	Number making stings	Total percentage causing injury	
									A[1]	B[2]
Lead arsenate, 2 lb. to 100 gal.	Mist	2	35			18	10	2	41.2	58.3
		2	50			42	21	2		
		3	50			44	23	1	48.0	54.5
		4	35			24	6	0	22.4	31.1
		4	50			37	13	0		
		Total	220	19.5	1.4	155	73	5	35.4	50.3
	Coarse	2	35			21	9	0	23.5	31.7
		2	50			42	9	2		
		3	55			49	6	3	14.3	17.6
		3	50			36	3	3		
		4	35			21	1	2	7.1	11.3
		4	50			32	2	1		
		Total	275	20.0	5.4	201	30	11	14.9	20.3
	Film	2	35			20	6	1	23.5	34.5
		2	50			38	10	3		
		3	55			49	6	3	13.3	17.3
		3	50			32	5	0		
		4	35			21	1	1	7.1	10.9
		4	50			34	1	3		
		Total	275	22.9	3.6	194	29	11	14.5	20.6
	Grand total		770	20.9	3.6	550	132	27	20.6	29.0
Lead arsenate, 8 lb. to 100 gal.	Mist	2	25			15	9	0	32.0	45.3
		2	50			38	13	2		
		3	25			22	9	0	26.7	32.6
		3	50			40	11	0		
		4	35			23	3	0	16.5	24.6
		4	50			34	11	0		
		Total	235	24.7	1.7	172	56	2	24.7	33.7
	Coarse	2	25			14	1	3	14.7	20.4
		2	50			40	5	2		
		3	25			22	2	2	10.7	13.6
		3	50			37	4	0		
		4	35			17	0	0	1.2	2.6
		4	50			22	1	0		
		Total	235	29.4	5.5	152	13	7	8.5	13.2
	Film	2	25			17	8	1	24.0	32.1
		2	50			39	6	3		
		3	25			19	4	0	10.7	16.7
		3	50			29	2	2		
		4	35			14	0	0	0.0	0.0
		4	50			20	0	0		
		Total	235	31.9	6.4	138	20	6	11.1	18.8
	Grand total		705	28.6	4.5	462	89	15	14.7	22.5
90%-10% sulfur-lead arsenate dust		2	50			37	9	2	18.0	24.0
		2	50			38	7	0		
		3	55			52	2	0	5.7	6.9
		3	50			34	2	2		
		4	35			19	0	0	0.0	0.0
		4	50			31	0	0		
		Total	290	22.1	4.5	211	20	4	8.3	11.4
Check		2	60			44	28	0	52.7	64.4
		2	50			46	30	0		
		3	55			51	18	0	42.8	46.4
		3	50			46	27	0		
		4	35			26	12	0	38.8	50.8
		4	50			39	21	0		
		Total	300	16.0	0.0	252	136	0	45.3	53.9

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[1] Based on number of larvae placed on sections of glass.

[2] Based on number of larvae transferred to apples.

of two, three and four hours, the larvae were transferred to untreated apples. Subsequently, determinations were made of the number of entrances and stings on the apples.

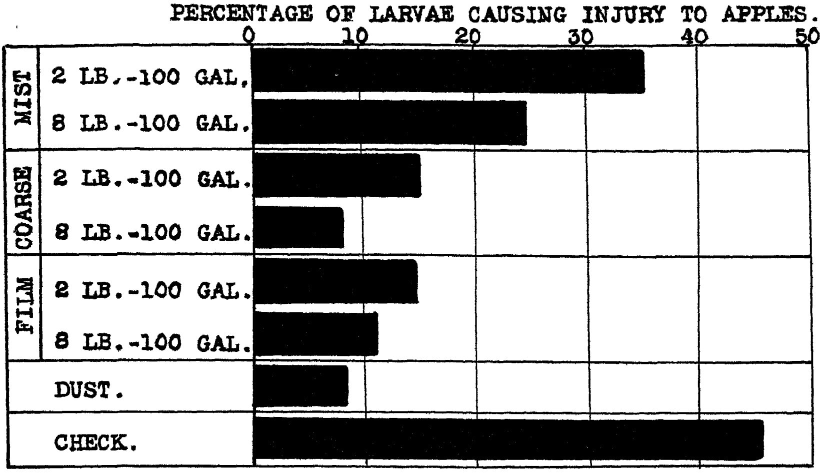

Fig. 16. Graph based on the data of table 13, indicating the extent that freshly hatched larvae were able to injure apples after crawling over sections of glass sprayed and dusted with lead arsenate.

Complete results of the tests are given in table 13 and a summary in table 14. The results are illustrated graphically in figure 16. More than twice as many larvae entered the apples from the mist coverage as from the coarse and film coverages. This inferiority of the mist coverage agrees with all other tests reported on foregoing pages. The coarse and film coverages gave about the same results.

TABLE 14

Summary of Data on Tests with Sections of Glass, Compiled from Results shown in Table 13

Type of coverage	Lead arsenate, 2 lb. to 100 gal.		Lead arsenate, 8 lb. to 100 gal.		90%-10% sulfur-lead arsenate dust		Check—untreated	
	Total injury—per cent		Total injury—per cent		Total injury—per cent		Total injury—per cent	
	A[1]	B[2]	A[1]	B[2]	A[1]	B[2]	A[1]	B[2]
Mist coverage	35.4	50.3	24.7	33.7				
Coarse coverage	14.9	20.8	8.5	13.2				
Film coverage	14.5	20.6	11.1	18.8				
Dust coverage					8.3	11.4		
Check							45.3	54.8
Average	20.7	29.0	13.3	22.5	8.3	11.4	45.3	54.8

[1] Percentage of injury based on the number of larvae placed on the sections.

[2] Percentage of injury based on the number of larvae transferred from the sections to the apples.

Seven per cent more larvae became poisoned on the sections sprayed with the eight-pound concentration than with the concentration of two pounds. The explanation for this result, the author believes, is that the thick deposits of the concentration of eight pounds to one hundred gallons stimulated the larvae to bite against the poison while this thigmotactic reaction was brought into play to less extent with the thinner deposits of the lower concentration. About the same percentage of larvae became poisoned on the dusted sections as on those having the coarse coverage of lead arsenate at eight pounds to one hundred gallons of water.

CALYX SPRAY STUDIES

Limited studies were made for the purpose of obtaining information on the efficacy of lead arsenate in preventing the entrance of larvae through the calyces of apples. Yellow Bellflower apples were used in the experiments. At the time the trees were in bloom, certain branches were tagged and the blossoms sprayed individually by means of a small bulb atomizer. In order to give uniform treatment to all blossoms, the tip of the atomizer was held approximately two inches directly in front of each calyx and four full aspirations made on the atomizer. This resulted in a finely divided spray striking the calyx with force and in sufficient quantity that some of the spray ran off the sepals. In case of the mist spray, the tip of the atomizer was held six inches from the calyx and the same number of aspirations made, but the spray reached the calyx only as floating mist particles. The following treatments were made:

Lead arsenate, 2 lb. to 100 gallons of water.
- (1) Full blossom, forceful spray; applied before petals had fallen.
- (2) Calyx, forceful spray; applied just after all petals had fallen.
- (3) Calyx, forceful spray with casein-lime spreader added at the rate of one pound to one hundred gallons of spray; applied just after all petals had fallen.
- (4) Calyx, mist spray; applied just after all petals had fallen.

Lead arsenate, 4 lb. to 100 gallons of water.
- (5) Calyx, forceful spray with casein-lime spreader as in No. 3; applied just after all petals had fallen.

The last week of July the apples that developed from the treated blossoms were picked and taken into the laboratory. Each apple was cut transversely into portions of one-third and two-thirds, the one-third portion being the blossom end. The cut surface of the blossom portion was placed on the bottom of a saucer and a small quantity of water placed in the saucer (fig. 17). Ten freshly hatched larvae were then placed inside the calyx, just under the sepals. Four days later the parts were removed and cut vertically so as to divide the calyx cavities into halves. Records were then made of the injuries. After this the sepals were cut off at their bases and thrown away. By use of a sharp knife, each one-half calyx cavity was cut out and determinations made by the Gutzeit method of the quantity of arsenic in the outer and the inner calyx cups. The stamens were included with the outer cup.

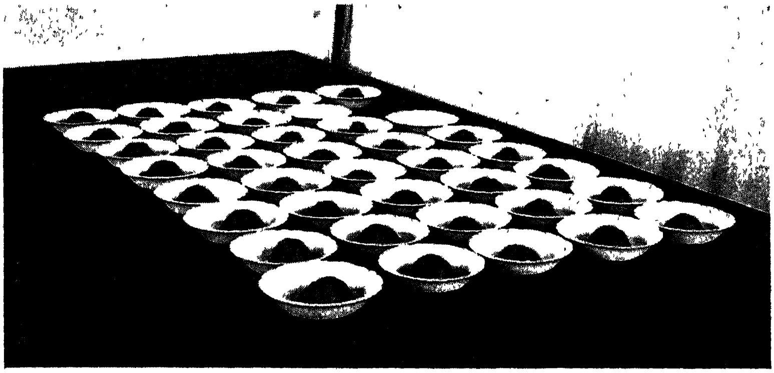

Fig. 17. The blossom ends of apples placed in saucers containing a small quantity of water for calyx spray studies.

TABLE 15

Results of Calyx Spray Tests in which Ten Larvae were Placed in Each Calyx Cavity

Number of test	Treatment	Number of calyces	Number of entrances		Average micromilligrams of As_2O_3 per calyx cup and cavity		
			Outer cups	Inner cups	Outer cups	Inner cups	Average per calyx cavity
1	Full blossom; lead arsenate, 2 lb. to 100 gal.	5	....	....	2.4	0.8	3.2
2	Calyx; lead arsenate, 2 lb. to 100 gal.	10	....	....	6.7	2.3	8.9
3	Calyx; lead arsenate, 2 lb. to 100 gal. and spreader	21	0	1	9.5	7.0	16.5
4	Calyx; mist; lead arsenate, 2 lb. to 100 gal.	11	....	....	1.0	0.6	1.6
5	Calyx; lead arsenate, 4 lb. to 100 gal. and spreader	12	1	4	20.0	13.3	33.3

The results of the tests are given in table 15.* The principal value of the studies is to indicate the relative effectiveness with which lead arsenate is placed in the calyx cavities by different spray methods, a matter that has been widely discussed during the past quarter century. The mist spray showed less than one-fifth as much arsenic per calyx cavity as the forceful spray. Almost twice as much arsenic occurred in the calyx cavities sprayed with lead arsenate and casein-lime spreader as in those sprayed only with lead arsenate. In applying the spray without the spreader it was noticed that there was a marked tendency for the particles of spray to bound off or to collect into drops and run off without wetting the calyces. Spraying before the petals had fallen resulted in about one-third less arsenic per calyx cavity than spraying after the petals had fallen.

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* Unfortunately, the data on the injury in tests 1, 2, and 4, were accidentally destroyed.

It is especially interesting to note that in test number 5, in which lead arsenate was used at the rate of four pounds to one hundred gallons of water, five larvae or approximately four per cent of the number transferred, entered apparently unharmed.

STUDIES ON THE BEHAVIOR OF FRESHLY HATCHED LARVAE

In order to understand the manner in which lead arsenate protects apples, it is necessary to observe the behavior of the larvae. Thigmotaxis, phototaxis and thermotaxis were the most important tropisms influencing the larvae in the studies reported in this paper.

Behavior on Unsprayed Apples

When a freshly hatched larva is placed on an apple, it crawls rapidly over the surface, apparently in search of a suitable place to make entrance. If the surface is smooth, the larva may crawl for two or three hours before making any attempt to dig into the apple. Usually after the first fifteen or twenty minutes, the rate of locomotion gradually decreases and frequent pauses may occur during which the head is thrown from side to side. In the latter movement numerous fibers are spun beneath the head. Occasionally larvae were observed to grasp these fibers with the thoracic legs and bring the tips of the mandibles into contact with the apple but seemingly without making any serious attempt to force the mandibles through the skin.

The larva exhibits a decided tendency to examine any slight eruption or other irregularity on the surface of the apple. When first placed on the apple, however, it may not pay the least attention to what may appear to the observer to be excellent places for entrance.

MANNER OF EFFECTING ENTRANCE

If no especially favorable point of entrance is encountered, the larva finally comes to rest and spins a mat of fibers, attached to the skin of the apple, beneath its head. This mat is grasped by the thoracic legs and the main strength of the body centered on forcing the points of the mandibles through the skin. The particles of skin that are cut out appear always to be rejected. The time required for making entrance varies greatly, according to the vigor of the larva, and light and temperature. One larva that was placed in direct sunlight just after starting an entrance, excavated a burrow deep enough to receive its body in twenty-four minutes. Usually the time required ranged from one to three hours when the temperature of the laboratory was about 76° F.

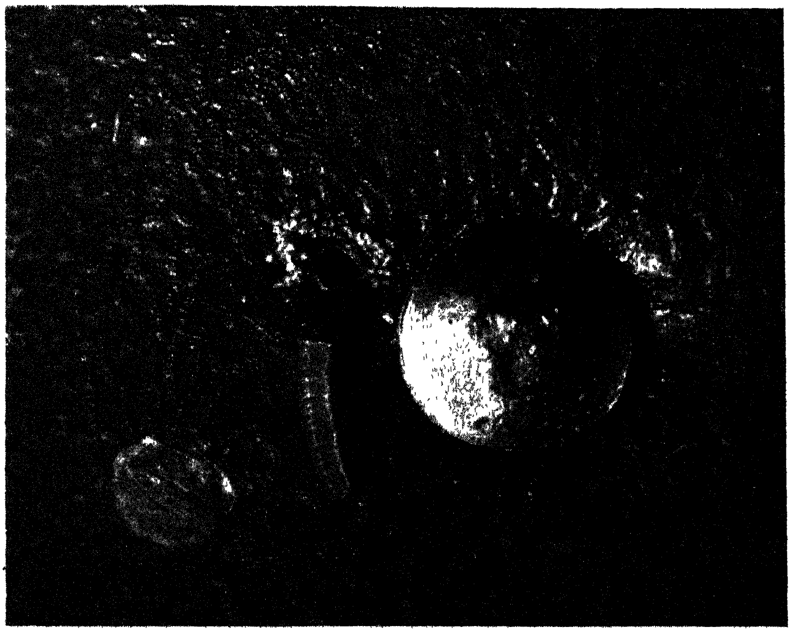

Fig. 18. Microphotograph of a newly hatched larva and an entrance which it had started. Bits of apple skin cut off by the larva are piled about the hole. On the right is the head of a pin and on the lower left the shell of a codling moth egg.

The fact that the larva rejects the apple skin was noted in 1897 by Card[31] and Slingerland,[32] but its significance has been almost entirely overlooked by later investigators. Two kinds of tests were made to determine whether the larva swallows any of the skin in digging into the apple. A small apple was given a thick coating of water-proof ink. After being suspended for a few days until the odor of the ink had disappeared, a number of larvae were placed on the apple. When a larva had burrowed the length of its body into the apple, it was removed and the digestive tract dissected out and examined for the presence of particles of ink. While in many instances the particles could be seen through the larva's body, accurate information required dissection. From the careful study of twenty-five larvae, the following data were obtained:

	Number of larvae	Per cent of larvae
Black particles of ink found in digestive tract	18	72.0
No particles of ink found in digestive tract	7	28.0

In the other test, three apples were coated with gentian violet stain, and larvae transferred and observed as in the above test. The following data were obtained:

	Number of larvae	Per cent of larvae
Stain pronounced in digestive tract	33	60.0
Stain slight in digestive tract	14	25.4
No stain in digestive tract	8	14.6

The studies indicated quite clearly that swallowing of particles of apple skin occurs incidentally as the larva digs into the apple. As previously noted herein, this has an important bearing on the efficacy of the film coverage of lead arsenate in protecting apples. If the film is thin, it might be expected that as many as fifteen per cent of the larvae will effect entrances without swallowing any poison. Since the larva must dig into the apple by use of its mandibles, it seems evident that the thicker the coating of poison is, the greater the chance that some poison will be swallowed.

BEHAVIOR ON SPRAYED APPLES

When placed on an apple having a spotted coverage of lead arsenate, the larva exhibits a slight tendency to rest upon the deposits of poison. In doing this, mats of fiber are sometimes spun on the deposits and a number of instances were observed in which the mandibles were brought directly into contact with the poison. Some poison may at times be swallowed in this reaction. Perhaps the most marked reaction is the thigmotactic response to the thick lower edges of spray deposits. Larvae were commonly observed to place the lower margin of their heads against the raised edges of deposits and spend several seconds or a few minutes "nosing" about. It was noticed that entrances and stings were commonly made at the edges of deposits. This is shown by the microphotograph in figure 19. Three entrances are shown, the lowest one being made directly through a deposit and the two toward the top being made at the edges of deposits. In order to obtain data on this question, an apple was sprayed with calcium carbonate in concentration of eight pounds to one hundred gallons of water, a coarse coverage being produced. Larvae were then transferred and after they had entered careful examination was made of the places where entrances were made. The following results were obtained:

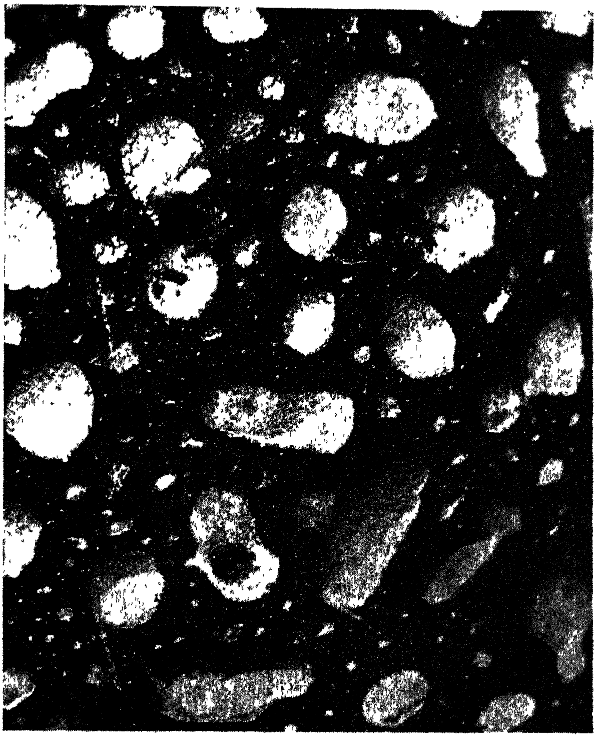

Fig. 19. Microphotograph of three entrances of freshly hatched codling moth larvae in apple with spotted coverage of lead arsenate.

	Number of entrances	Number of stings	Per cent of total injury
Through deposits	1	5	19.3
At edges of deposits	12	2	45.2
Between deposits	10	1	35.5

BEHAVIOR ON DUSTED APPLES

Loose dust very decidedly interferes with the movement of larvae over apples. The greater protectiveness of the dust covering of lead arsenate, as shown by foregoing tests, apparently is due to the larvae becoming poisoned while merely crawling over the dusted surface.

Whether poisoning occurs through the particles of lead arsenate being taken into the spiracles or being swallowed, has not been definitely determined. An experiment was performed in which a small quantity of cornmeal was soaked in water-proof ink. After drying for several days, allowing the odor to disappear, the meal was ground in a mortar and the finest part separated out and dusted over an apple. Larvae were then placed on the apple and at the end of one-half hour their digestive tracts were examined for the presence of particles of cornmeal. Of twenty-five larvae examined, three revealed definite evidence of having swallowed some particles.

SUMMARY AND CONCLUSIONS

Probably no insect pest in the history of horticulture has been the subject of as much discussion and experimentation as the codling moth. During the past forty-five years investigations have dealt to a very large extent with arsenical sprays as a means of control. The extensive literature reveals many incongruous experimental data and varied beliefs. Spray experiments have been confined almost entirely to tests made under orchard conditions.

Laboratory studies with freshly hatched codling moth larvae were undertaken for the purpose of ascertaining the more basic facts relating to the efficacy of lead arsenate in protecting apples against codling moth injury.

The principal tests were made with apples taken from trees during July and August and sprayed in a technical manner in the laboratory. Four types of spray coverages were differentiated and tested: mist, coarse, overspray and film.

The spray was relatively ineffective in protecting the apples. With the concentration of two pounds of powdered lead arsenate to one hundred gallons of water, approximately one-third of the larvae entered the apples unharmed through the coarse, overspray and film coverages.

The mist coverage was very much less effective than the other coverages.

The coarse, overspray and film coverages were about equal in protectiveness at concentrations of four pounds or less of lead arsenate to one hundred gallons of water. At concentrations of more than four pounds to one hundred gallons the film coverage gave greater protection than the other coverages.

Increasing the concentration, in all coverages, resulted in decidedly decreasing the percentage of larvae effecting entrances.

At equal amounts of arsenious oxide per square centimeter of apple surface, approximately one-third as many larvae entered through the film coverage as through the coarse and overspray coverages.

In all coverages, protectiveness varied directly with the amount of arsenious oxide per square centimeter of apple surface.

Apples heavily oversprayed and then lightly shaken to cause the large drops of spray to run off were injured very much more than oversprayed apples that were not shaken.

The percentage of larvae making stings increased as the percentage effecting entrances decreased except that at the concentration of sixteen pounds of lead arsenate to one hundred gallons of water there was a decrease both in stings and entrances.

A loose, light covering of 90%–10% sulfur-lead arsenate dust was much more effective in protecting the apples than lead arsenate spray in concentration of two pounds to one hundred gallons.

Experiments in which larvae were placed on sprayed and dusted sections of glass and later transferred to unsprayed apples revealed that many larvae became poisoned while crawling over the sprayed and dusted glass; that the mist coverage was decidedly inferior to the others in killing efficiency; that the greater the concentration of spray and the more lead arsenate on the glass, the more larvae killed; and that the loose covering of sulfur-lead arsenate dust was high in effectiveness.

Experiments in which larvae were placed on the leaves of cuttings from the growing ends of apple branches, to which apples were artificially attached, revealed that the spray on the leaves and bark was about equal to the spray on the apples in destroying the larvae and that the percentage of larvae poisoned varied directly with the concentration of the spray.

The thick lower edges of spray deposits stimulate the thigmotactic reactions of freshly hatched larvae.

Tests in which larvae were placed on apples coated with water-proof ink and on apples covered with gentian violet stain indicated that some larvae may reject the skin to such extent as not to swallow any in digging into the apple.

Thickness of film is the most important factor relating to the protectiveness of the film coverage.

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* The manuscript for this paper was prepared in the spring of 1925. These references have since been appended because they include discussions of the experimental data with special reference to the application of the data to the practical control of the codling moth in apple orchards.

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UTILIZATION OF THE SOILS IN THE GILROY REGION

STANLEY W. COSBY

INTRODUCTION

> Care must be taken to observe the winds
> And changing skies, what modes and habits be
> The region's heritage, what gifts each place
> Bears or denies. These acres favor corn
> In yonder vines grow better; elsewhere spring
> Fruit orchards and a wealth of unsown green.
>
> —*Virgil.*

It was recognized early in agricultural history that individual crops do not grow equally well in all environments—that certain plants require definite climatic, soil, and other conditions for their maximum development.

Also, in regions containing diverse soils and growing diverse crops, it has been observed that under continued agricultural development there is a tendency, due to economic factors, for crops to be planted and to persist in soils where they are profitable and to disappear from soils where they are not. Eventually, the crops of a region will become aligned with the soils on which they are economically best suited.

In the south-central portion of the Santa Clara Valley a relatively long continued development of intensive agriculture has occurred, in which soil variation has been the chief factor in crop-distribution. The trees, vines, and other crops in this region are located apparently with regard to these variations. (Figure 1.) So marked is this tendency that the crop and soil boundaries frequently coincide.

This study deals with the extent of the correlation of these crops and soil types and, in this region, the proportion of each crop on each soil has been assumed to be a measure of the relative suitability of that crop for that soil.

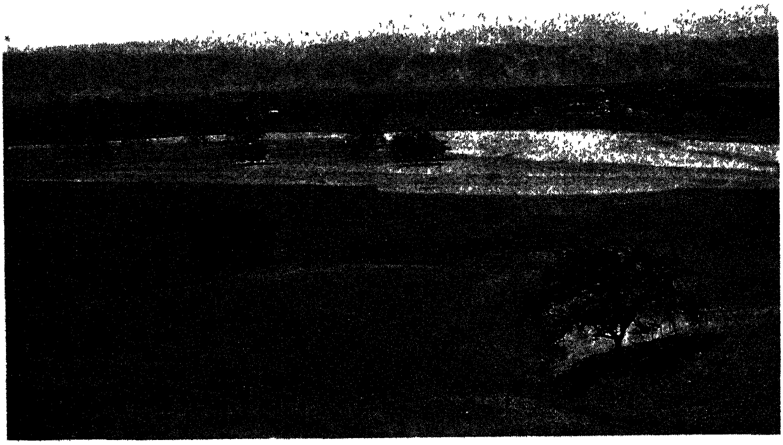

Fig. 1. Looking westerly across Santa Clara Valley near Coyote. Grazing on hills in foreground; grain for hay on the coarse soils along the Coyote Creek; orchards (mainly French prunes) on the medium-textured Yolo soils in middle distance; and sugar beets, truck and seed crops on the heavy Dublin soils at base of the distant hills.

DESCRIPTION OF THE GILROY REGION

Location.—This study covers that part of the Santa Clara Valley which extends from the vicinity of Coyote southeasterly to Gilroy and from the lower slopes of the Diablo Range on the east to the foothills of the Santa Cruz Mountains on the west. As indicated in figure 2, this region is roughly rectangular in shape with a length of twenty miles and an average width of three.

Topography and Drainage.—The foothills of the mountain ranges on the east and west are sharply defined, emphasizing the relatively flat and smooth valley plain which slopes with a gentle gradient from an elevation of about 450 feet above sea-level at the mouth of the Coyote Creek canyon to approximately 250 feet at Coyote and 200 feet at Gilroy. Near Morgan Hill an inconspicuous drainage divide is formed across the valley by the alluvial fan of Coyote Creek, which is the major stream in the northern drainage basin. Llagas Creek, with its tributaries, carries the southern drainage into Monterey Bay, by way of the Pajaro River. Neither topography nor drainage have had a determining influence on the distribution of crops in this region.

Climate.—The climate of the Gilroy Region is characterized by a long, practically rainless summer season and a rainy winter period. The U. S. Weather Bureau reports the mean annual temperature at Gilroy as 58.4° F. and the average annual precipitation as 20.09 inches.

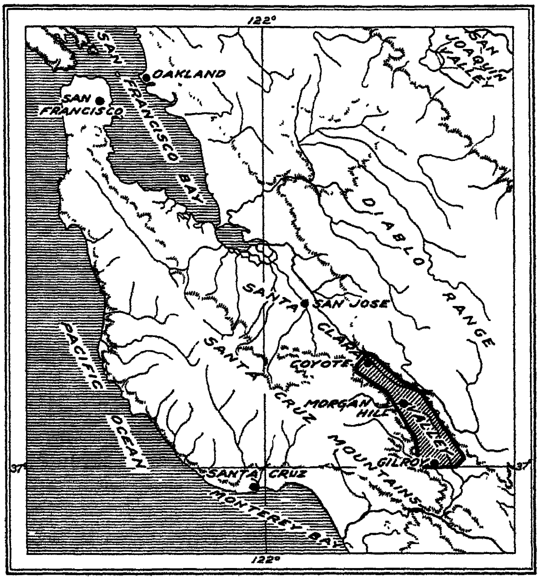

Fig. 2. Sketch map of the Santa Clara Valley and vicinity showing the location of the Gilroy Region.

There is little variation in climate between different points in the region; Coyote, on the north, has temperature and rainfall similar to those for Gilroy in the southern part, while Morgan Hill with a similar temperature has a slightly higher precipitation, probably about 22 inches. From an agricultural standpoint, this difference in rainfall is of very minor importance, as irrigation is commonly practiced throughout the region.

Acting as barriers, the Santa Cruz Mountains on the west and the Diablo Range on the east protect the region from the cold ocean fogs of the coast and the desiccating heat of the interior valley. With this protection from either extreme the climate of this portion of the Santa Clara Valley is a moderate one, conducive to the profitable production of a wide range of crops.

Transportation Facilities.—The coast route of the Southern Pacific Company traverses the length of the Gilroy Region and supplies shipping facilities to all coast and eastern markets. No point in the region is more than four miles from a station on this railroad. The Coast Highway (a concrete-paved main highway connecting San Francisco and Los Angeles) parallels the railroad and, with the network of connecting roads, provides an adequate system for vehicular traffic.

Soils.—Detailed descriptions of the soils of this region may be found in the Soil Survey of the Gilroy, California, Area (Advance Sheets, Field Operations of the U. S. Bureau of Soils, 1923), the Reconnoissance Soil Survey of the San Francisco Bay Region, California (Field Operations of the U. S. Bureau of Soils, 1914), and other California surveys. For the purpose of this paper, brief references to their major characteristics will suffice.

Three main classes of soils are represented in the Gilroy Region; (*a*) Recent alluvial, (*b*) Old transported, and (*c*) Residual. They are formed of material originating in the rocks of the coast mountain ranges, which consist principally of sandstones and shales with minor amounts of chert and numerous intrusions of basic igneous character. On the basis of their general physical characteristics, these soils may be arranged in seven sub-groups, as follows:

> (1) Recent alluvial soils of light to *medium texture,* deposited under conditions of adequate drainage. These are deep, friable, well-drained soils of medium to high fertility and include the light to medium-textured types of the Yolo and Vina series.
>
> (2) Recent alluvial soils of *heavy texture,* generally deposited under conditions of restricted drainage. Characteristically, these have an adobe structure, a very high water-holding capacity, and are of high fertility. This sub-group includes the heavy-textured types of the Yolo, Dublin and Conejo series.
>
> (3) Recent alluvial soils which are being deposited at a sufficiently slow rate to permit progressive weathering, causing *heavier subsoils,* which tend to retard the movement of water and limit the development of root systems. In this sub-group are the Honcut and Laguna series of soils.
>
> (4) Old transported soils of gravelly character having relatively *permeable subsoils.* These soils possess a lower content of organic matter and are of somewhat lower fertility than the recent alluvial soils. This sub-group includes the soils of the Pleasanton and Corning series.

(5) Old transported soils having non-calcareous, heavy-textured, compact, and relatively *impervious subsoils,* which limit the root-system development and impede the movement of water. This includes the soils of the Rincon, Pinole, and San Ysidro series.

(6) Old transported soils having medium to heavy-textured surface soils and slightly compact, *calcareous subsoils.* This sub-group is of minor importance in the Gilroy Region and includes the limited areas of soils belonging in the Antioch and Montezuma series.

(7) Residual soils; formed in place by the weathering of underlying bedrock. Their typically shallow depth and hilly topography makes them of minor importance. In this sub-group are the soils of the Aiken, Olympic, Arnold, and Climax series.

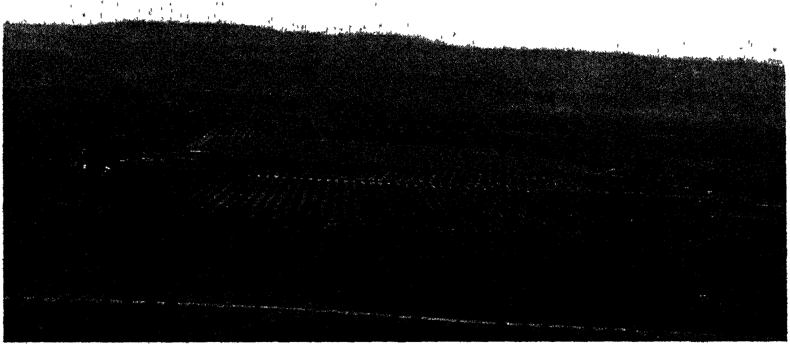

Fig. 3. View across valley between Morgan Hill and Madrone, showing character and extent of agricultural development.

Agriculture.—Gaspar de Portolá, who discovered the valley in 1769, described it as "a beautiful park-like region spotted with magnificent oaks and abounding in wild game." The earliest permanent settlements were made during the first quarter of the nineteenth century and until California came under the jurisdiction of the United States the chief activity was the raising of cattle. Grain, principally wheat, was the first major crop to be planted, although attention soon was directed to dairies, orchards, and vineyards. In 1870, the coast route of the Southern Pacific Company was constructed through the valley, giving a further impetus to agricultural development, which forced the cattle interests to the bordering foothill districts. Since 1880 the planting of grains and other extensive crops has been rapidly replaced by fruit growing, a transition which has caused this region to be at the present time one of the most highly developed agricultural districts of the state. (Figure 3.)

Approximately 85 per cent of the Gilroy Region may be classed as tillable land, of which about 80 per cent is cropped or otherwise developed. Orchards (predominantly of the French prune) and vineyards comprise the greater part of the plantings. Irrigation is extensively practiced, nearly 90 per cent of the orchards and about two-thirds of the entire planted area being irrigated. Water for this purpose generally is pumped from wells.

CROP-SOIL MEASUREMENTS AND CORRELATIONS

Crop and soil data were collected during three growing seasons, 1923, 1924 and 1925. By means of a special survey in July, 1924, the crops were identified, measured and plotted on a suitable base map. This was superposed on a map of the soils (from the Gilroy soil survey) and a combined soil-crop map constructed (plate 1). The individual and the aggregate acreages of each class of crop on each type of soil were determined from this map and tabulated. (Plate 2.)

Of the 47,045.1 acres covered by this study, these data show that 39,747.0 acres are tillable and that 26,375.8 acres of these are planted to crops of various classes. Planting does not occur on all of the types and phases of soil in like proportion; Climax clay adobe (with an extent of 562.3 acres) is entirely unplanted, while approximately 91 per cent of the Dublin silty clay loam (1751.8 acres in extent) is planted to crops.

Smaller differences in relative development occur on the three most important and extensive soil types. The Yolo silt loam (with a total area of 4240.8 acres) has 86 per cent of its area planted to crops, the Pleasanton gravelly loam (with 7257.0 acres) has 82 per cent planted to crops, and the Pinole silt loam (5604.9 acres in extent) has 78 per cent of its area planted.

Planted, unplanted, and total acreages for each of the thirty-one soil types and phases and the three miscellaneous classes of materials recognized in the Gilroy Region are given in table 1.

The crops of the region are divided into twenty-two classes, eight of which are separated into "bearing" and "non-bearing." In most cases the mixed, or interplanted, crop areas are distinguished according to their components. The French prune is the most extensively planted crop, occupying nearly 17,000 acres, as compared to about

TABLE 1

PLANTED AND UNPLANTED ACREAGES ON SOIL TYPES

Soil type, or phase	Planted area, in acres	Unplanted area, in acres	Total area, in acres
Subgroup (1):			
Yolo gravelly loam	991.1	460 3	1451.4
Yolo fine sandy loam	330 0	726.5	1056.5
Yolo silt loam	3671.1	569.7	4240.8
Yolo silt loam, shallow phase	1502.8	510.0	2012.8
Yolo silty clay loam	1637.2	227.6	1864.8
Vina gravelly loam	574.5	407.9	982.4
Vina silt loam	15.1	15 4	30.5
Subgroup (2):			
Yolo clay adobe	283.8	79.7	363.5
Dublin silty clay loam	1584.6	167.2	1751.8
Dublin silty clay loam, gravelly phase	205 1	118.0	323.1
Dublin clay adobe	979.2	1014.3	1993.5
Conejo clay loam	581.9	523.9	1105.8
Conejo clay adobe	1235.6	1937.0	3172.6
Subgroup (3):			
Honcut clay loam	150.4	32.4	182.8
Honcut clay loam, gravelly phase	84 7	58.1	142.8
Laguna fine sandy loam	194.6	82.3	276.9
Laguna loam	40.8	85 3	126.1
Subgroup (4):			
Corning loam	93.3	56.4	149.7
Pleasanton gravelly loam	5994 3	1262.7	7257.0
Subgroup (5):			
Pinole silt loam	4420 5	1184.4	5604.9
Pinole silt loam, rolling phase	240.6	250.3	490.9
San Ysidro silt loam	820.5	112.2	932.7
Rincon loam	15 5	64.4	79.9
Rincon loam, rolling phase	84.7	188.9	273.6
Subgroup (6):			
Antioch clay loam	42 3	722.3	764.6
Montezuma clay adobe	12.8	582.8	595.6
Subgroup (7):			
Arnold loam	174 1	264.4	438.5
Aiken gravelly clay loam	241.6	264.9	506.5
Olympic gravelly clay loam	130.5	556.8	687.3
Olympic clay adobe	43.4	282.8	326.2
Climax clay adobe	....	562.3	562.3
Total, soil types	26,375.8	13,371.2	39,747.0
Miscellaneous:			
Riverwash	....	66.7	66.7
Rough broken land	4.2	498.9	503.1
Rough mountainous land	79.5	6648.8	6728.3
Total, miscellaneous	83.7	7214.4	7298.1
Grand total	26,459.5	20,585.6	47,045.1

4500 acres of grapes, more than 1000 acres each of Sugar prunes, apricots, alfalfa, truck and seeds, and about 400 acres of pears. The other crops, with few exceptions, are of minor significance in this study and vary in extent between 12 and 700 acres.

The different crop-classes in the Gilroy Region and the extent of their pure, mixed and total planted acreages are shown in table 2.

TABLE 2

PURE, MIXED AND TOTAL PLANTED AREAS OF EACH CROP CLASS

Crop	Pure planting, in acres	Mixed planting, in acres	Total planting, in acres
French prunes	13,533.5	3,079.8	16,613.3
Sugar prunes	1,161.7	695.4	1,857.1
Apricots	1,197.5	204.5	1,402.0
Peaches	396.9	231.8	628.7
Pears	220.6	188.5	409.1
Cherries	5.5	18.4	23.9
Apples	13.0		13.0
Figs		13.0	13.0
Walnuts	99.8	541.7	641.5
Almonds	75.6	107.9	183.5
Mixed orchard	220.3	7.2	227.5
Grapes	2,309.7	2,101.2	4,410.9
Alfalfa	1,037.8	10.5	1,048.3
Truck or seeds	904.6	123.7	1,028.3
Sugar beets	629.2		629.2
Tomatoes	393.6	318.1	711.7
Corn or sorghum	155.0	291.0	446.0
Strawberries	51.1	21.0	72.1
Bushberries	19.0	8.0	27.0
Myrobalan seedlings	61.2		61.2
Eucalyptus planting	34.8		34.8
Nursery stock	12.0		12.0
Total acreage	22,532.4	7,961.7*	30,494.1*

* The net area of mixed planting is 3926.7 acres and the net total planting is 26,459.1 acres, there being a duplication of 4035.0 acres in the mixed planting which appears in these totals.

Although some of the crops in this region are grown on a large number of soil types, a very noticeable relationship exists between the more extensive crops and soils. This is more obvious if comparisons are made on the basis of the proportion of the planted area of each soil which is occupied by the crop.

The French Prune.—The most extensively planted crop in the Gilroy Region is the French prune, 16,613.3 acres being occupied by this fruit either in uniform stand or interplanted with other crops.* Cultural methods are simple and chiefly featured by irrigation, clean cultivation, and light pruning. Although a green cover crop is frequently grown, applications of commercial fertilizers are extremely rare. The chief rootstock used for the French prune is the myrobalan.

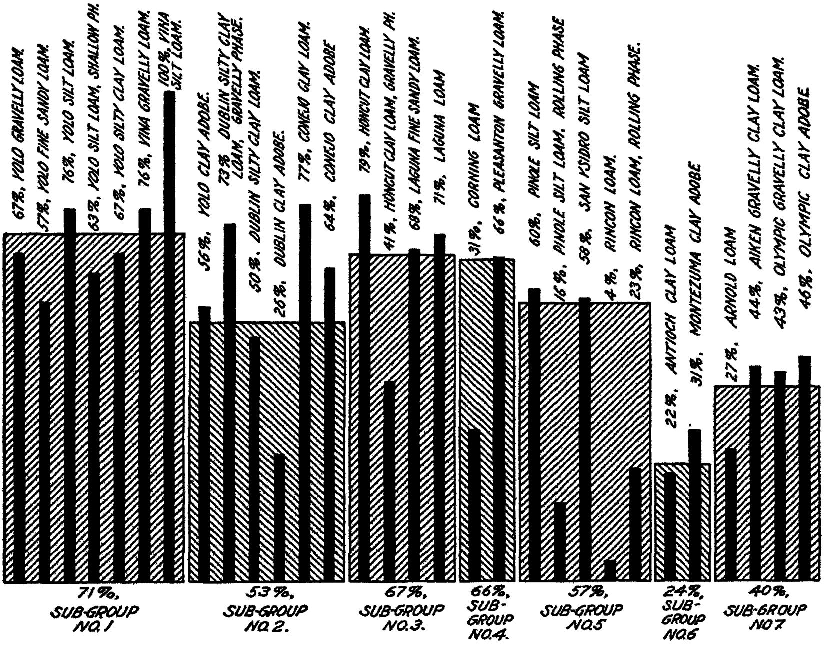

FIGURE 4

RELATIVE DISTRIBUTION OF THE FRENCH PRUNE

The heights of the black, narrow columns indicate the percentages of the cropped area of each soil type planted to French prunes.

The heights of the cross-lined, wider blocks indicate the percentages of the cropped area of each sub-group of soil planted to the French prune.†

Each sub-group of soils is composed of those types having the same general physical characteristics, see page 458. These are briefly as follows:

Sub-group no. 1 includes the recent alluvial soils with medium texture.
Sub-group no. 2 includes the recent alluvial soils of heavy texture.
Sub-group no. 3 includes the recent alluvial soils with heavy subsoils.
Sub-group no. 4 includes the old transported soils with permeable subsoils.
Sub-group no. 5 includes the old transported soils with impervious subsoils.
Sub-group no. 6 includes the old transported soils with calcareous subsoils.
Sub-group no. 7 includes the residual soils.

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*Included in this crop class, but having no appreciable influence on the major relationships, are very minor acreages of Imperial, Robe de Sergeant, Silver, Standard and Italian prunes.

† In calculating the percentages for the sub-groups only those soil types having a part of their acreage planted to this crop were included.

For more than half a century the French prune has been grown commercially in this region. The total acreage has shown a steady increase during this period, although many of the earlier orchards in unsuitable locations have been replaced by other crops. At present by far the greatest acreage of French prunes is located on the Yolo, Pleasanton and Pinole series of soils. This crop occupies 60 per cent of the total acreage of the Yolo, 54 per cent of the Pleasanton and about 43 per cent of the Pinole series of soils.

A comparison on the basis of the percentage of the planted area of the different soils (figure 4) shows that the three most extensive soil types have the following relationship: French prunes constitute 76 per cent of the planted area of the Yolo silt loam, 66 per cent of the Pleasanton gravelly loam and 60 per cent of the Pinole silt loam. This decreasing proportion is also found to occur in a comparison of the three sub-groups of soil in which these types are placed. Sub-group no. 1 has 71 per cent of its planted area in French prunes, sub-group no. 4 has 66 per cent and sub-group no. 5 is the lowest with 57 per cent of its planted area so utilized. A greater significance is given to these relative plantings if they be considered in conjunction with those for the Sugar prune which follow.

The Sugar Prune.—Among the orchard crops, the Sugar prune ranks next to the French prune in extent of planting, occurring on 1857.1 acres of the Gilroy region. Although this represents only slightly more than 10 per cent of the acreage occupied by the French variety, its importance lies in the relatively extensive planting on the soils of the Pinole series.* Cultural methods are similar to those for the French prune, with the exception of pruning. The Sugar prune is more heavily pruned than the French under similar circumstances. This practice is most extreme in those orchards located on the deep, friable soils of the Yolo and Vina series where the Sugar prune is cut back in the severe manner typically used with the apricot.

The Sugar prune was introduced by Luther Burbank at the close of the last century in an attempt to develop a prune having a larger size with the excellent qualities of the French variety. This later introduction has been the chief reason for the relatively smaller acreage planted to this variety as compared to the French prune. Apparently due to a greater capability to endure adverse conditions and to a more vigorous habit of growth, the Sugar prune is better able to withstand the limiting effects of the heavy and impervious subsoils of the Pinole and related series than is the French prune. In fact, most orchardists consider the Sugar prune to be more satisfactory on these soils than they are on the deep, friable soils of the Yolo and Vina series.

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* Investigation of numerous references to the "Sugar prune soils" of this region has shown that the Pinole series of soils have been so-named locally; indicating that the local growers have found a significant relationship between these soils and the Sugar prune.

The Sugar prune is most extensively planted on the Pinole silt loam, occurring on 665.9 acres of this type (approximately 35 per cent of the total area devoted to this crop), as compared with 347.7 acres on the Pleasanton gravelly loam and 77.3 acres on the Yolo silt loam.

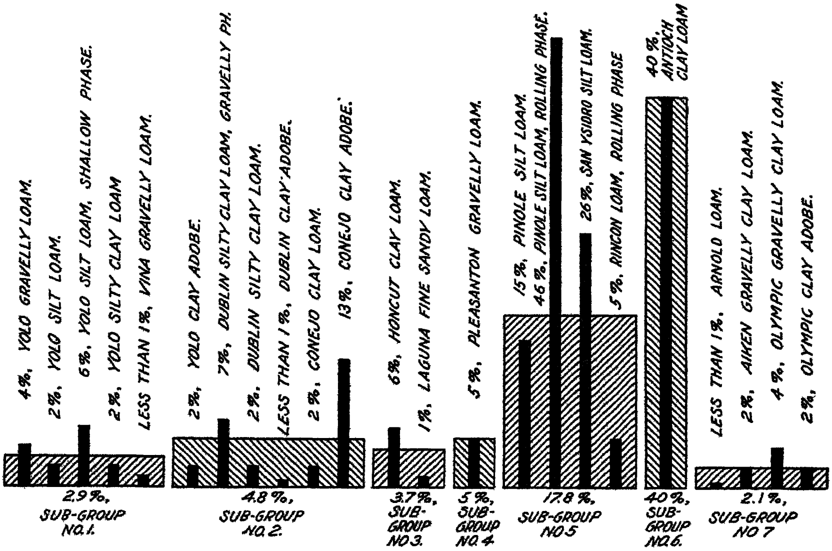

FIGURE 5

RELATIVE DISTRIBUTION OF THE SUGAR PRUNE

The heights of the black, narrow columns indicate the percentages of the cropped area of each soil type planted to Sugar prunes.

The heights of the cross-lined, wider blocks indicate the percentages of the cropped area of each sub-group of soil planted to the Sugar prune.*

Each sub-group of soils is composed of those types having the same general physical characteristics, see page 458. These are briefly as follows:

Sub-group no. 1 includes the recent alluvial soils of medium texture.
Sub-group no. 2 includes the recent alluvial soils of heavy texture.
Sub-group no. 3 includes the recent alluvial soils with heavy subsoils.
Sub-group no. 4 includes the old transported soils with permeable subsoils.
Sub-group no. 5 includes the old transported soils with impervious subsoils.
Sub-group no. 6 includes the old transported soils with calcareous subsoils.
Sub-group no. 7 includes the residual soils.

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* See footnote p. 463.

On a basis of the percentage of planted area (figure 5), the three most extensive soil types show the following relationship: 2.0 per cent of the crops planted on the Yolo silt loam and 5.0 per cent of the planted area of the Pleasanton gravelly loam are Sugar prunes, as compared to 15.0 per cent in the case of the Pinole silt loam. A comparison of the sub-groups of soils containing these three types shows a similar trend; sub-group no. 1 has less than 3.0 per cent of its planted area in Sugar prunes, sub-group no. 4 has 5.0 per cent, and sub-group no. 5 has nearly 18.0 per cent.

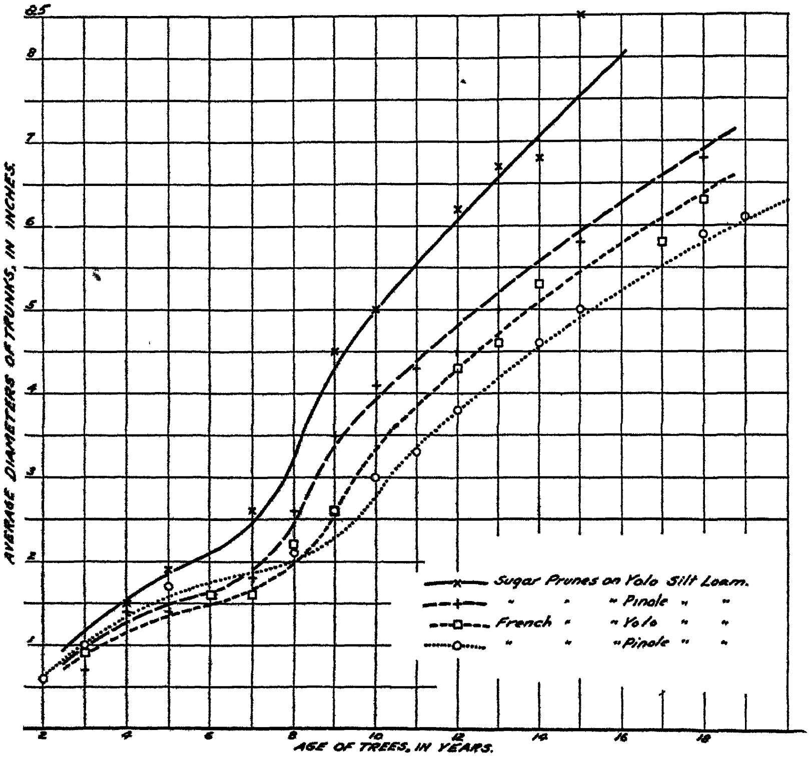

Fig. 6. Curves of average trunk diameters for French and Sugar prune trees of various ages growing on the Yolo and Pinole silt loams.

These figures show a distinct contrast in the relative distribution of these two varieties of prunes, particularly in the case of the Yolo and Pinole silt loams.

Growth Measurements of French and Sugar Prunes.—The successful production of French and Sugar prunes on the Pinole and Yolo silt loams being apparently associated with differences in habits of growth and vigor, four hundred trees of these two varieties growing on both soils were measured to determine: (1) rate of increase of trunk diameters, and (2) average length of shoot growth at different ages.

The average diameters of the trunks of these two prune varieties were found for trees of various ages on the two soils and curves were constructed of the rate of increase (figure 6). On the basis of these curves, each of the varieties made a more rapid growth on the Yolo silt loam than they did on the Pinole silt loam. The trunk measurement of the ten-year old Sugar prune trees on the Yolo silt loam is one and one-half times that of the French prune on the same soil and nearly twice the size of that variety when grown on the Pinole silt loam. Further, the diameters of the bearing Sugar prunes (eight years and older) on the Pinole silt loam average about one-half inch larger than do those of the French prunes of similar age on the Yolo silt loam, ordinarily considered a better orchard soil.

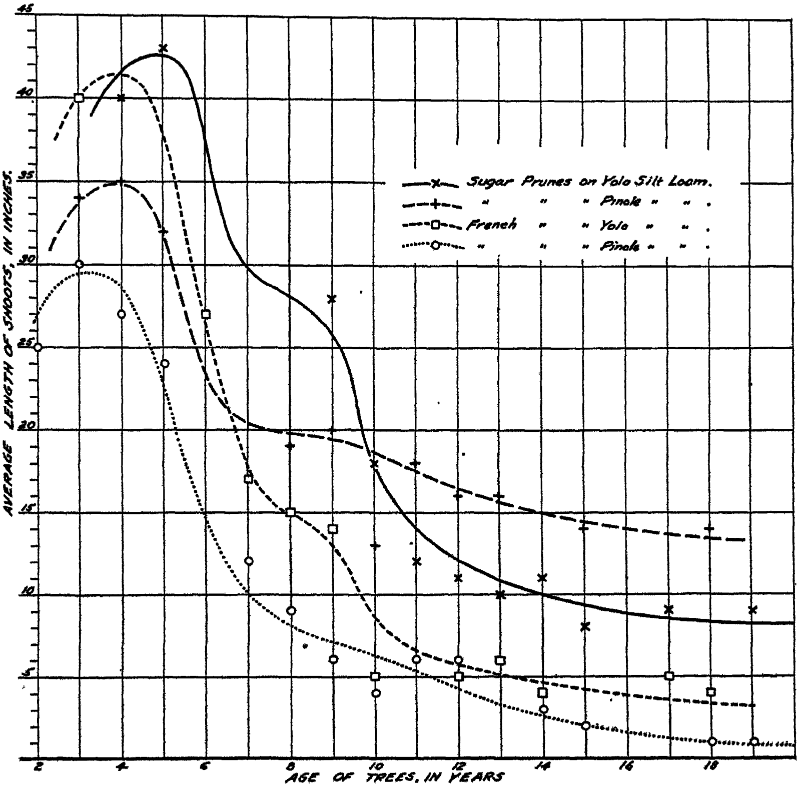

Fig. 7. Curves of average shoot-growth for French and Sugar prune trees of various ages growing on the Yolo and Pinole silt loams.

In a discussion of the effects of the severity of pruning on the rate of trunk growth of young fruit trees, one investigator* states that the severe pruning of young trees results in a smaller diameter increase than does light pruning. Applying this conclusion to these two prune varieties, the increased size of the Sugar prune trunks may be ascribed to a greater inherent vigor of growth and not to pruning practices, as the Sugar prune is pruned more heavily than the French, particularly when grown on such soils as the Yolo silt loam.

Curves also were constructed of the average lengths of shoot growth for trees of various ages of these varieties growing on both soil types. (Figure 7.)

From these measurements it may be concluded that the sugar prune has a greater inherent vigor of growth than the French prune and that either variety makes a more extensive growth on the Yolo silt loam than on the Pinole silt loam. A striking difference is shown in the length of growth made by the bearing Sugar prunes on the Pinole silt loam as compared to that made by the French variety on the same soil (figure 8). These figures, supplemented by numerous field observations, indicate that the most desirable and profitable growth of these two prunes occurs when the French prune is planted on the Yolo silt loam and the more vigorous growing Sugar prune is located on the Pinole silt loam.

The Grape.—The grape is the second most extensively planted crop in the Gilroy Region, occupying 4410.9 acres, comprising about 16 per cent of the total planted area. This is about one-quarter the extent of the French prune acreage and more than twice that of the Sugar prune.

With the sole exception of one very small planting of Thompson Seedless near San Martin, the grape plantings are all of the wine varieties. Despite the uncertain future for this crop, few vineyards have been replaced by other crops in recent years, and a normal proportion of the present acreage consists of new plantings. Under ordinary circumstances the greater part of the vineyards are not irrigated, but during the dry season of 1924 more than half of the acreage received one or more applications of water. The growers have found the grape to be better suited to the old transported soils than to the deep soils of recent alluvial formation. On the latter soils the vines make a very heavy vegetative growth which undesirably shades the fruit, adds to the labor of harvest, and seldom results in an increased yield, while the quality of the juice is generally poorer.

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* W. P. Tufts. Univ. California Agr. Exp. Sta. Bull. 313:111–153. 1919.

The grape is most extensively planted on the Pleasanton gravelly loam, 1703.2 acres being so utilized, while the Pinole silt loam ranks second with 1261.6 acres and the Yolo silt loam third with 339.5 acres.

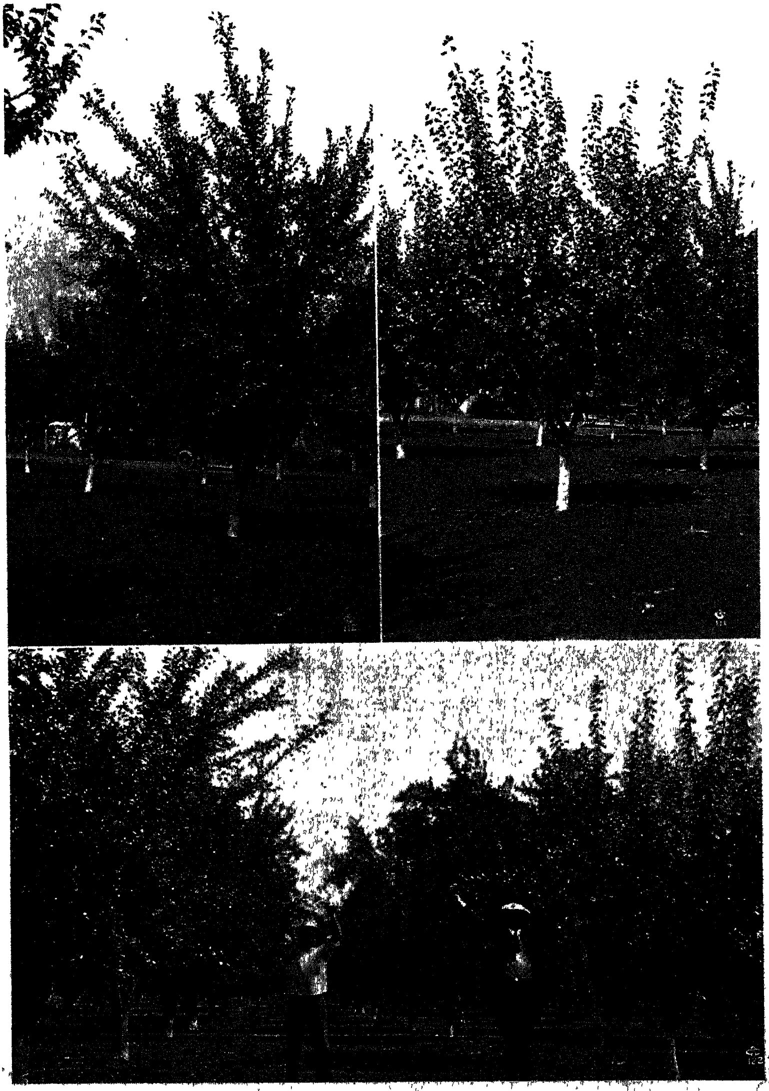

Fig. 8.—Ten year old orchard east of Gilroy showing difference in length of shoot growth between French (tree No. 2) and Sugar (tree No. 1) prunes on Yolo silt loam. Length of 1925 growth shown between outstretched hands of the two men in lower photo.

On the basis of percentage of the planted area of the soil types (figure 9), the Pleasanton gravelly loam and the Pinole silt loam show similar relations, with the grape comprising 28 and 29 per cent of the cropped acreage of these soils. With a single exception, the Laguna loam, the recent alluvial soils have a considerably lower proportion planted to grapes. The Laguna loam, like a few other soil types which show conspicuously large percentages planted to the grape, is of limited significance due to the minor area involved. A corresponding relation is shown by the three principal sub-groups of soils; sub-group no. 1 has 7.7 per cent of its planted area in grapes, sub-group no. 4 has 28.0 per cent and sub-group no. 5 has 27.2 per cent so utilized.

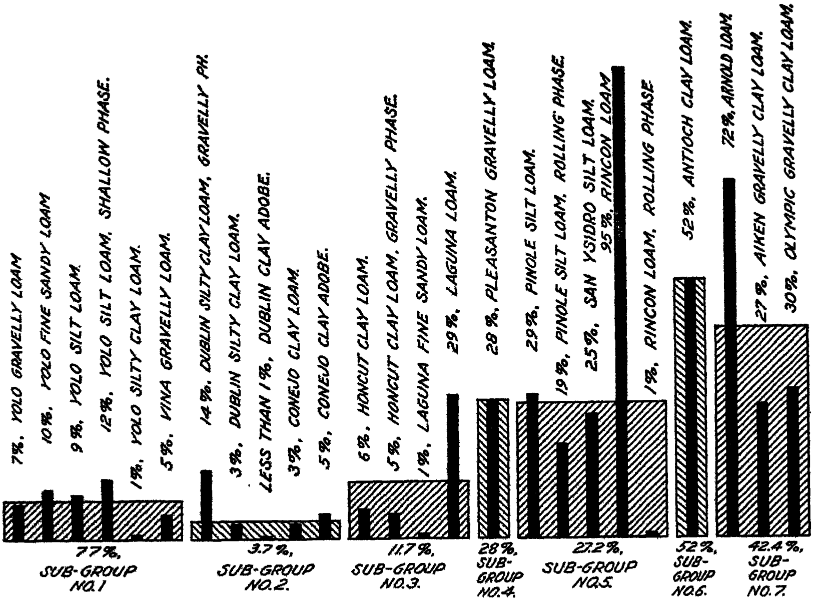

FIGURE 9

RELATIVE DISTRIBUTION OF THE GRAPE

The heights of the black, narrow columns indicate the percentages of the cropped area of each soil type planted to grapes.

The heights of the cross-lined, wider blocks indicate the percentages of the cropped area of each sub-group of soil planted to the grape.*

Each sub-group of soils is composed of those types having the same general physical characteristics, see page 458. These are briefly as follows:

Sub-group no. 1 includes the recent alluvial soils of medium texture.
Sub-group no. 2 includes the recent alluvial soils of heavy texture.
Sub-group no. 3 includes the recent alluvial soils with heavy subsoils.
Sub-group no. 4 includes the old transported soils with permeable subsoils.
Sub-group no. 5 includes the old transported soils with impervious subsoils.
Sub-group no. 6 includes the old transported soils with calcareous subsoils.
Sub-group no. 7 includes the residual soils.

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* See footnote p. 463.

The correlation of this crop with the Pleasanton gravelly loam and the Pinole silt loam is in accord with the observations of the various growers as to the most profitable and desirable soil situation for the grape.

The Pear.—The pear (with a total acreage of only 409.1 acres) shows a significant distribution. Although grown on a number of

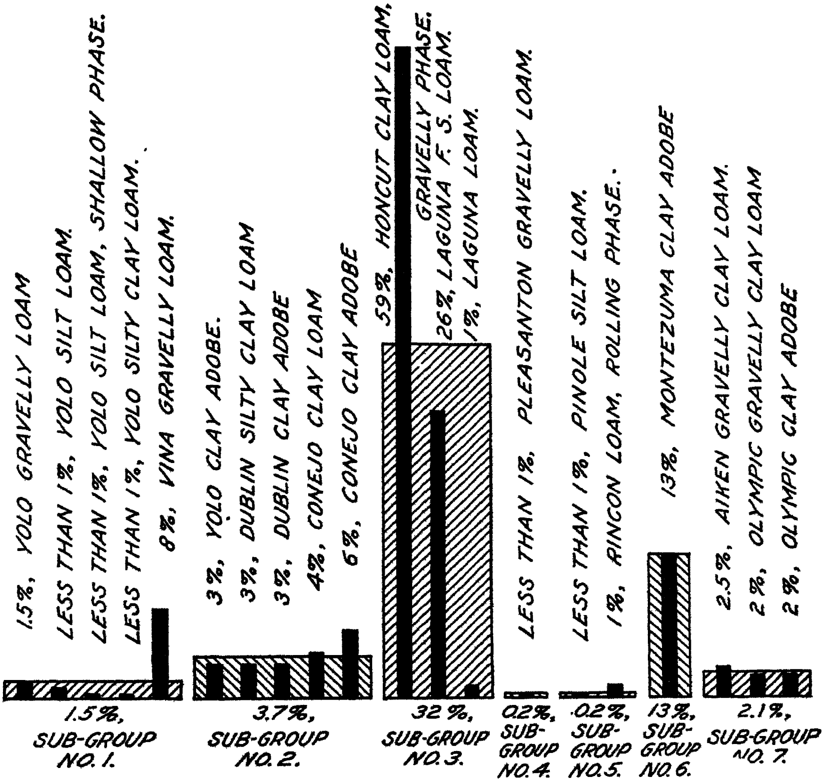

FIGURE 10

RELATIVE DISTRIBUTION OF THE PEAR

The heights of the black, narrow columns indicate the percentages of the cropped area of each soil type planted to pears.

The heights of the cross-lined, wider blocks indicate the percentages of the cropped area of each sub-group of soil planted to pears.*

Each sub-group of soils is composed of those types having the same general physical characteristics, see page 458. These are briefly as follows:

Sub-group no. 1 includes the recent alluvial soils of medium texture.
Sub-group no. 2 includes the recent alluvial soils of heavy texture.
Sub-group no. 3 includes the recent alluvial soils with heavy subsoils.
Sub-group no. 4 includes the old transported soils with permeable subsoils.
Sub-group no. 5 includes the old transported soils with impervious subsoils.
Sub-group no. 6 includes the old transported soils with calcareous subsoils.
Sub-group no. 7 includes the residual soils.

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* See footnote p. 463.

soils in the Gilroy Region, this crop has been planted mainly on the heavier-textured recent alluvial soils included in the second and third sub-groups.

The Bartlett is the chief variety. The Japanese pear is used as the principal root-stock in all but the oldest plantings. Cultural methods are simple and practically all of the orchards are irrigated.

More than 90 per cent of the acreage planted to pears is on the recent alluvial soils, particularly those of the second and third sub-groups. The most extensive planting of this fruit (70.3 acres) is on the Conejo clay adobe, with slightly lesser acreages on the Laguna fine sandy loam and gravelly phase of the Honcut clay loam. The three most extensive soil types, Yolo silt loam, Pleasanton gravelly loam, and Pinole silt loam have only very minor acreages planted to this crop.

On the basis of the percentage of the planted area of the soil types (figure 10), the gravelly phase of the Honcut clay loam has 59 per cent planted to pears, the Laguna fine sandy loam has 26 per cent, Vina gravelly loam has 8 per cent, and then next occur* the five soil types included in sub-group no. 2. A comparison of the soil sub-groups indicates a similar relationship; sub-group no. 1 has 1.5 per cent (102.0 acres) of its planted area in pears, sub-group no. 2 has 3.7 per cent (173.0 acres), and sub-group no. 3 has 32 per cent (102.0 acres) planted to this fruit.

This correlation of the pear plantings and the heavy-textured recent alluvial soils is in accord with the opinions of growers, who have found that the pear is the most profitable orchard crop in the region for these soils. It is difficult to account for the relatively small percentage shown by sub-group no. 2, as compared to that of sub-group no. 3, except on an economic basis; only a very limited number of the crops of the region appear to grow well on the Honcut and Laguna soils although the second sub-group is particularly adapted to the production of seed and truck crops (see later) as well as being suited to a number of other crops grown in this region.

Truck and Seed Crops.—The production of vegetable seeds is one of the unique and important agricultural activities of the Gilroy Region. The Santa Clara Valley is estimated to furnish 95 per cent of the lettuce seed, nearly all of the radish seed, and about 75 per cent of the onion seed produced in the United States. All of the factors that are involved in making this section one of the ideal spots for the production of these highly specialized crops can not be successfully determined, although the absence of wind and rain during the growing season is one of the most important causes. Within the district, however, the distribution of the seed crops is definitely associated with the occurrences of distinct soil characteristics. The nature of this crop being such that irrigation is very undesirable, the growers have limited their plantings to the heavier types of the Yolo and Dublin series of soils, the highest in water-holding capacity and among the most fertile in the Gilroy Region.

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* In this consideration the Montezuma clay adobe has been ignored, despite its 13 per cent, as the acreage involved is extremely small.

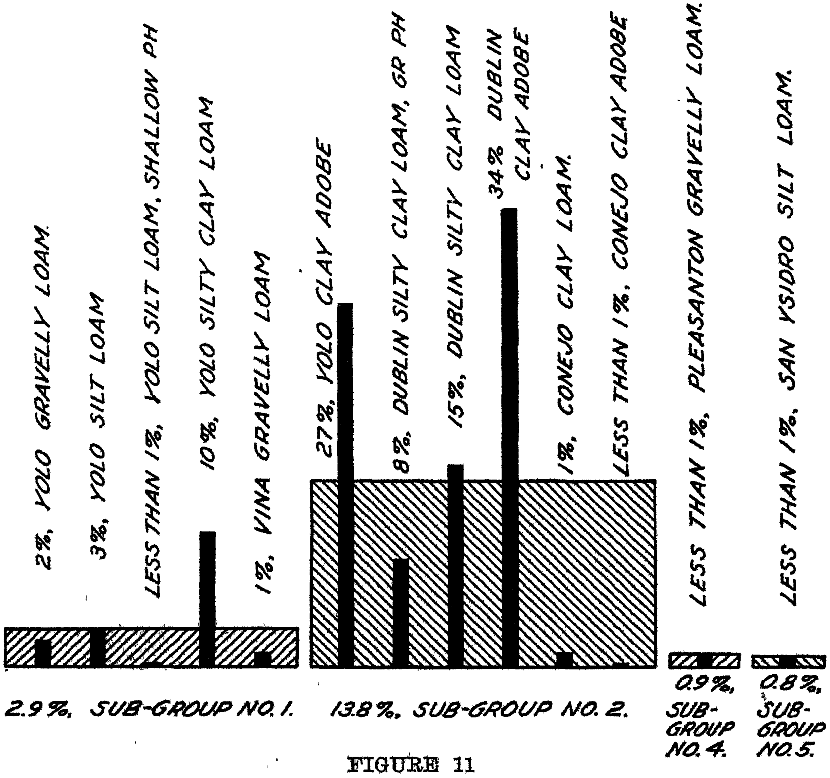

FIGURE 11

RELATIVE DISTRIBUTION OF TRUCK OR SEED CROPS

The heights of the black, narrow columns indicate the percentages of the cropped area of each soil type planted to truck or seeds.

The heights of the cross-lined, wider blocks indicate the percentages of the cropped area of each sub-group of soil planted to truck or seeds.*

Each sub-group of soils is composed of those types having the same general physical characteristics, see page 458. These are briefly as follows:

Sub-group no. 1 includes the recent alluvial soils of medium texture.
Sub-group no. 2 includes the recent alluvial soils of heavy texture.
Sub-group no. 4 includes the old transported soils with permeable subsoils.
Sub-group no. 5 includes the old transported soils with impervious subsoils.

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* See footnote p. 463.

Limited acreages of vegetables for market purposes are grown in this region, but at the time the crop survey was made it was impossible to determine whether the crop was destined for market as fresh vegetables or as seed, in some cases the owner himself had not made a decision. For this reason the vegetable plantings were included with the seed crops under the common heading of "Truck and Seed Crops."

Lettuce and onion seed have been found to produce best on the clay adobe and silty clay loam of the Dublin and Yolo series, while the radish yields best on the slightly lighter-textured soils, such as the Yolo silty clay loam and silt loam.

The most extensive acreage of truck and seed crops is on the Dublin clay adobe, 331.3 acres (nearly one-third of the total acreage of the crop) occurring on this type. The next important plantings are the 236.4 acres on the Dublin silty clay loam and the 169.6 acres on the Yolo silty clay loam.

Comparing the distribution of this crop on the basis of the percentage of planted area of each soil type (figure 11), the Dublin clay adobe is again seen to be the most important, with 34 per cent of its planted area so utilized, followed in order by the Yolo clay adobe, with 27 per cent; the Dublin silty clay loam, with 15 per cent; and the Yolo silty clay loam, with 10 per cent. On the basis of a percentage of the planted areas of the sub-groups a similar relationship is shown; sub-group no. 2 has 13.8 per cent of its planted area utilized for the production of truck and seed crops as compared to 2.9 per cent of sub-group no. 1 and less than 1.0 per cent in the case of sub-groups no. 3 and no. 4.

The foregoing correlations indicate that the truck and seed crops in the Gilroy Region are grown almost exclusively on the recent alluvial soils of heavy texture, which are most economically suited to their production.

Alfalfa.—Alfalfa is of extensive occurrence in this region, with an aggregate extent of 1048.3 acres. The distribution of this crop is of minor significance as a large portion of the total acreage occurs in small plantings; many of the orchardists use it as a ground cover in their fruit-drying yards. A few large fields have been planted commercially in the central and southern portion of the Gilroy Region and these occur most extensively on the recent alluvial soils of the Yolo and Dublin series.

The Apricot.—The apricot is one of the extensively planted crops of the region, having a total extent of 1402.0 acres. However, its distribution is also of limited significance as frost hazard, rather than soil suitability, is frequently the determinant factor. The greater acreage of the mature trees are located on the level floor of the valley, south of Coyote, where the earliest orchard development occurred. Many of the earlier plantings of apricots in that locality have been replaced by orchards of French prunes. The greatest number of young apricot orchards, as well as the largest acreage, is situated on the upper portions of the alluvial fans and terraces on the eastern side of the valley where the air drainage is better and frost damage lessened. The apricot is most extensively planted on the Yolo series (470.0 acres), with lesser acreages on the Pinole (about 300 acres) and Pleasanton series (about 200 acres).

The Peach.—The peach is a crop of minor importance in the Gilroy Region, having an extent of 628.7 acres. A much larger acreage of this fruit was planted here formerly, but a large part of its acreage has been replanted to prunes and other more profitable crops. At present only 20.5 acres of non-bearing peaches occur in this region, a fair indication of the amount of interest which the growers are giving this crop.

The lighter-textured, more gravelly soils are said to produce the best peach for drying purposes, while the medium-textured alluvial soils produce a better canning fruit. More than 50 per cent of the peaches (328.9 acres) occur on the Pleasanton gravelly loam, nearly 20 per cent (113.9 acres) are on the Yolo silt loam, and about 15 per cent (101.9 acres) on the Pinole silt loam.

The Walnut.—The walnut is a more extensively planted crop in the region than is apparent to the casual observer. In addition to a large number of trees planted in borders, or individually, there are 641.5 acres planted in groves. By far the greater portion of these occur interplanted in orchards, only 99.8 acres being pure groves of walnuts.

Although the walnut has been found to be most profitable on the deep, fertile, recent alluvial soils, there are extensive plantings on the old transported soils of the valley floor. This has been done not through any belief that these soils are best suited to the walnut but as an attempt to avoid the disastrous effects of the oak root fungus which attacks the prune and similar trees.

The plantings of walnuts on the Pleasanton gravelly loam (207.1 acres) is the most extensive, but the distribution of this crop is of only minor importance in this study, as in many of the plantings the factor of soil suitability has been superseded by other considerations.

Tomatoes.—Tomatoes are one of the important "cash" crops interplanted in the young orchards. Of the 711.7 acres of tomatoes planted on the soils of the region, nearly four hundred acres are pure plantings and 253.1 acres are in young orchards of prunes, pears and figs.

The mixed plantings have little significance in these studies, as the orchard is generally the more important from the grower's point of view. A greater significance lies in the distribution of the pure plantings of this crop; the most extensive occurrences being on 111.0 acres of the Dublin silty clay loam, 57.0 acres of the Dublin clay adobe and 48.1 acres of the Yolo silty clay loam. This correlation is in accord with the findings of the growers that the tomato is most profitable on the heavy-textured, fertile, recent alluvial soils.

Sugar Beets.—There are 629.2 acres of sugar beets in the region, all located in the north near Coyote. The importance of these plantings lie in the character of the soils on which they occur; more than 90 per cent of the crop being grown on the bodies of the calcareous phase of the Dublin clay adobe and silty clay loam. The moderate to high content of lime found in these bodies of soil, which is not found in the Dublin clay adobe and silty clay loam in other portions of the region, makes them better suited to the production of the sugar beet.

Minor Miscellaneous Crops.—A number of other crops are being grown in the Gilroy Region, but due to their minor occurrence their distribution is of limited, or no, significance in this study. These include the cherry, apple, fig, almond, strawberries, bushberries, corn and sorghum, as well as very limited acreages of seedling myrobalan, nursery stock, and eucalyptus groves.

CONCLUSIONS

The foregoing data indicate that definite correlations exist between the various types of soil and the crops occurring in the Gilroy Region and that the distribution of the major crops has been determined by soil variation. These correlations have resulted from "trial and error" methods of planting during a relatively long period of agricultural development.

A direct comparison on an acreage basis is unsatisfactory, as variation occurs in the crop acreages and in the extent of the different soil types. A more significant relationship, one in which due weight is given to the less extensive occurrences, is shown by the percentages of the total planted area of each soil occupied by the various crops.

The French prune, the most extensively planted crop in the Gilroy Region, occurs on 76 per cent of the planted area of the Yolo silt loam, 66 per cent of the Pleasanton gravelly loam, and 60 per cent of the Pinole silt loam. The significance of these figures is in the relative order of their occurrence on these three most important soil types and not in the comparative narrow range of percentages.

In the case of the Sugar prune this order is reversed. The Sugar prune occupies only 2 per cent of the planted area of the Yolo silt loam and 5 per cent of the Pleasanton gravelly loam as compared to 15 per cent of the Pinole silt loam. Measurements of shoot growth and trunk diameters, as indices of relative vigor, support the conclusions of the growers that the Sugar prune is better suited to the Pinole silt loam and that the French variety is better suited to the Yolo silt loam.

The grape, the second most extensive crop, has been found to be more satisfactory on the old transported soils than on the deep, fertile, recent alluvial soils. These data show that the distribution of the grape in this region reflects this relationship as the crop occupies only 9 per cent of the planted area of the Yolo silt loam, although it occupies 28 per cent of the planted area of the Pleasanton gravelly loam and 29 per cent of the Pinole silt loam.

The other crops of the region, although of smaller extent, also are distributed predominantly on those soil types to which they are best suited. The pear occurs mainly on the heavy-textured recent alluvial soils, particularly those of the Laguna series. The apricot, probably the only crop of the region whose distribution has been affected more or less by climatic conditions, is planted most extensively on the soils of the Yolo series. Truck and seed crops are planted almost exclusively on the heavy types of the Yolo and Dublin soils, the highest in water-holding capacity and among the most fertile of the region. The pure plantings of tomatoes likewise occur predominantly on these soils. The sugar beet, although of limited extent, is practically confined to the calcareous phases of the heavy-textured Dublin soils. The other minor crops of the region show more or less correlation with the soil types, but are of doubtful significance due to their limited acreages.

This study is an evaluation of the effect of the soil type as shown by the distribution of the varieties of cultivated plants grown upon these types. It gives the combined results of a great number of environmental features, physical, chemical and biological, embodied within the zone occupied by the plant roots, without attempting to segregate any particular feature of soil difference which might influence this distribution. It evaluates the sum of all of these influences by measuring the crop distribution in a region where cropping has been continued for a long enough time so that through repeated failures or successes the cultivated crops have migrated to the soils which seem to provide most satisfactory conditions for their profitable production. In this it is comparable to a study of the natural distribution of plants in a virgin country.

As a result of these studies it appears proper to conclude that, *in a district such as the Gilroy Region the proportion of each crop on each soil is a measure of the relative suitability of that crop for that soil.*

An extension of these studies to other districts would determine the degree of suitability existing between standard crops and the other extensive soil types. This information would be an important guide in the determination of what crops should be recommended for the soils in regions where development is still in an early stage.

map

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CERTAIN WATER RELATIONS OF THE GENUS PRUNUS*

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This paper is based on a comparative study of stomatal behavior and moisture content of trees of the genus *Prunus* during the rainless summer months in California, where they are grown under conditions of both abundant and scanty soil moisture.

The behavior of stomata in relation to transpiration has been a riddle to physiologists. Lloyd,[15-16] working with *Fouquiera splendens* in Arizona, first stated that the regulatory effect of stomata on transpiration was almost nil. Later he modified this view and showed that transpirational losses followed stomatal opening. Other physiologists thought that, except for the small water loss due to cuticular transpiration, the stomata controlled the transpirational losses. Francis Darwin,[3] Knight,[10-11] and others studied the action of stomata by means of the porometer. This device consisted of a hollow receptacle fastened to the leaf, through which a stream of air was drawn. From the amount of air which could be drawn through a leaf under carefully controlled conditions, these workers drew their conclusions regarding the transpiration of the plant. The value of this method was problematical and Darwin and Pertz[4] stated that "it is not certain that we shall ever be able to deduce the size of stomata from readings of the porometer." Later, however, Darwin[3] showed that the parallelism between transpiration and stomatal aperture held within certain limits with *Hedera helix* and *Prunus*

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Laurocerasus in the moist climate of England. Balls,[1] working with the stomatograph on cotton under tropical conditions in Egypt, found that the stomata in response to light opened quickly to a maximum about 9 A.M. and thereafter closed rapidly. Laidlaw and Knight[12] studied excised stems of various plants by means of the porometer and reported a temporary opening of stomata before permanent closing when wilted. Lloyd[16] did not find this "preliminary opening." Knight[11] later reported that the stomata seemed to continue to open after transpiration fell off. This confirmed some of Lloyd's[16] results.

With regard to the relation of stomatal opening to water content of Gossypium leaves, Lloyd[17] reported that the "opening of stomata is accompanied by a net loss of water by the leaf, more being given off by transpiration than can be obtained to replace it." The same author further reported that the amount of water relative to the dry weight of the leaf decreased until noon or some time thereafter and then increased until 4 A.M. Livingston and Brown[13] reported that the moisture content of leaves of various desert plants in Arizona fell to a minimum during the period 1 P.M. to 5 P.M. and then rose to normal at 7 P.M. They further stated that "non-stomatal retardation of water loss appears to be continuously active until well into the night, its effects becoming mingled with those of stomatal closure at or about sunset. It appears that in the hours just preceding sunrise stomatal retardation seems to be alone manifest." Edith Shreve[18] found with *Parkinsonia microphylla* that the curve of stomatal behavior followed the relative transpiration curve in such a manner that the existence of an interrelation was evident.

Gain[7] showed many years ago that transpiration was affected by the water content of the soil. With regard to the influence of the soil, Edith Shreve[18] reported that the "maxima for relative transpiration . . . . were found to vary directly with the soil moisture." Dole[6] has recently stated that the "amount of available soil water has an important bearing on the rate of transpiration." Gray and Peirce[8] reported that "the factor regulating both food manufacture and stomatal opening is light." Their data tended to show that available soil moisture was also an important factor in stomatal regulation.

One of the most important reports on the action of stomata is that of Loftfield,[14] who worked with a great variety of plants mostly under semi-arid conditions. He reported the maximum of leaf turgor about midnight. He also stated that the stomata of certain plants opened at night in response to moonlight. According to this author, the opening of stomata in nearly all plants was correlated with light when conditions of soil moisture were favorable. When these conditions became unfavorable, the influence of light was decreased and in some cases nullified. He divided the herbaceous plants with which he worked into three general groups according to the behavior of their stomata during the day and night under favorable and unfavorable conditions.

The cereals, in which there was no opening of stomata at night, regardless of the amount of day opening, were placed in the first group. The amount and duration of day opening was dependent upon sunlight, evaporation, temperature, and water content of the leaves. In the second group, of which alfalfa was a member, the stomata normally were open during the day but closed all night. Under unfavorable conditions of soil moisture, however, the stomata of this group showed varying degrees of day closing and night opening. In the third group, which was typical of potatoes and beets, the stomata were normally open all day and all night. If conditions of soil moisture became critical, the stomata tended to remain closed all day and to open at night. Loftfield also worked with the apple, pear, peach, and sweet cherry. He placed these trees in the same general group with alfalfa, showing no midday closure or night opening under favorable conditions. Loftfield found no midday closure of stomata in fruit trees, even when this condition was observed in alfalfa. He attributed this lack of day-time closing to two facts: first, that there was a balance of water on hand in the trunks and branches of the trees, and second, that there was available moisture within reach of their extensive root systems.

Certain aspects of the general problem of behavior of stomata were emphasized by some of these workers. Darwin,[3] Knight,[11] and others pointed out the difficulties involved in connecting stomatal movement and transpiration as determined by the porometer or similar apparatus, Lloyd[15] and Loftfield[14] studied the behavior of stomata of many plants by the absolute alcohol method, and showed the influence of such factors as light, temperature, and soil moisture on stomatal movement.

Livingston,[13] Lloyd,[17] and others showed certain relations between water content and stomatal movement of leaves of certain xerophytic plants, while other workers called attention to the relation of soil moisture to the stomatal movement. In studies on the latter part of the problem the soil moisture conditions were usually not carefully controlled, and the data obtained were[4] not conclusive.

STATEMENT OF THE PROBLEM

The wilting of plants such as may be observed on any hot day is usually attributed to the fact that more water is lost by transpiration through leaves and other green parts of the plant than is absorbed by the roots and conveyed to the different parts. Thus, wilting is usually most pronounced during the afternoon, although evidence of it may often be seen quite early in the morning. Wilting, then, can be said to be due to a reduction of water content of the tissues of the plant below the amount necessary to maintain turgor in the cells.

Mature orchard trees supplied with ample water by means of irrigation are distinctly different in appearance from unirrigated trees, particularly during the latter part of the growing season. Lack of available moisture is apparent to any one familiar with orchard trees. Previous investigations have shown that stomata are intimately connected with transpiration even though the opening and closing of the stomata did not always seem to be directly correlated with the increase and decrease of transpiration. Various workers have shown that transpiration is regulated to a certain extent by the amount of moisture available in the soil. The behavior of stomata on leaves of fruit trees growing in soil containing available moisture, therefore, should differ from the behavior of stomata on similar trees growing in soil containing little or no available moisture. Series of experiments were carried out to see if such a difference of behavior did exist.

The second point investigated was that of the moisture content of the various tissues of the tree under the conditions previously described. Leaves of trees growing under conditions favorable for transpiration and for the opening of stomata, show a fluctuation in water content. The cohesion theory of the rise of water which is based upon the theory that water in the plant tissues is under tension indicates that the fluctuation occurring in the leaves after transpiration begins should be transmitted back to the trunk and roots fairly rapidly. Investigations were carried out at Davis and at Delhi in 1925 to see if such diurnal fluctuation in the water content of peach trees could be found.

METHODS

The leaves were stripped, killed, fixed, stained with Congo Red, and mounted with balsam according to Lloyd's[15] method. The width of the stomatal openings was measured by means of a micrometer eye-piece. The stomatal dimensions as given in the tables are the average measurement of ten stomata which seemed typical of all the stomata in the sample. In most cases samples were taken every hour. In some of the later experiments samples were taken every two and, in a few cases, every three hours.

The meteorological data included air temperature, relative humidity and notes on cloudiness, wind velocity, and presence of dew. In a few cases atmometer readings were also taken. Soil moisture was determined with a special soil tube. Soil samples were taken each time leaf samples were taken. The soil was sampled to a depth of six feet; the first sample from 0 to 3 feet, and the second from 3 to 6 feet. Each sample weighed about 500 grams. All samples were taken in duplicate or triplicate. The moisture equivalent, wilting coefficient, and hygroscopic coefficient were determined after the method of Briggs and Shantz[2] modified by Veihmeyer, Israelsen, and Conrad.[20] No attempt is made in this paper to discuss the various questions which have been raised regarding the significance or determination of these factors. In this paper, the amount of moisture below the theoretical hygroscopic coefficient was considered as unavailable to the tree. The amount of moisture between the hygroscopic coefficient and the wilting coefficient was considered as being moisture that was secured with difficulty by the tree. In other words, the tree roots were able to reduce the percentage of moisture in the soil below the wilting coefficient, but could not secure from this moisture enough water for the cells to regain turgidity.

Soils.—The soils at Mountain View and at Davis are similar, being classed by the Soil Survey as "Yolo fine sandy loam" and as "Yolo clay loam." The Mountain View soil, however, contained a little more gravel than the Davis soil. The soil at Delhi was classed as an "Oakley and Madera fine sand, undifferentiated," underlaid with a compacted subsoil at a depth of 5 to 6 feet. The moisture equivalent for the Mountain View soil was determined to be 22 per cent. The wilting coefficient and hygroscopic coefficient were 11.9 per cent and 8.05 per cent, respectively. The moisture equivalent of the Delhi soil varied from 5.8 per cent to 13.8 per cent. The wilting coefficient varied from 3.2 per cent to 7.5 per cent, and the hygroscopic coefficient from 2.1 per cent to 5.0 per cent. At Davis the moisture equivalent varied from 12.8 per cent to 29.3 per cent. The wilting coefficients varied from 6.8 per cent to 16.0 per cent, and the hygroscopic point from 4.6 per cent to 10.8 per cent. Because of the sandy nature of the Delhi soil, the relative amount of available moisture was small and lack of moisture was more readily detected by the appearance of the trees than was the case either at Davis or Mountain View.

The water table at Davis was approximately 18 feet below the surface during the time the samples were being taken. If the trees obtained any moisture from this source, it was apparently not sufficient in amount to affect the stomatal behavior. The depth of the water table at Delhi was not determined, but from data from a well near by it was safe to assume that standing water was not encountered closer than 30 feet beneath the surface. From data secured by Veihmeyer* it was found that mature prune trees in a loam soil extract all available soil moisture to a depth of six feet fairly rapidly, and to a depth of twelve feet before the end of the growing season. It seems reasonable to assume that the mature peach trees at Davis behaved in the same way. The presence of a compacted layer of soil about six feet beneath the surface at Delhi makes it probable that moisture below this level did not affect the behavior of the peach trees.

Meteorological Conditions.—Meteorological conditions at Davis and at Delhi are similar, and are typical of the interior valleys of California. The temperature during the day often reaches 100°F. and may go above 100°F. for several days in succession. The maximum temperature is usually between 85°F. and 95°F. The days are usually cloudless although occasionally light clouds persist until 8 or 9 A.M. and sometimes begin to form during the late afternoon. The relative humidity during the hot part of the day often goes as low as 30 per cent. The climate at Mountain View is typical of the central coast region. High fog or clouds often persist until 10 A.M. and the temperature rarely exceeds 85°F. during the hottest part of the day. During certain stages of the so-called "storm movements," the temperature may reach 100°F. and the relative humidity may drop to as low as 30 per cent, although during the greater part of the day it is from 60 to 75 per cent. During the afternoon there is usually a breeze from the San Francisco bay which lowers the temperature and increases the relative humidity. The differences in climatic conditions between Mountain View and Delhi are sufficient to bring about marked differences in the fruit industry of the two sections.

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* Unpublished.

The trees used in the experiment at Mountain View were mature Blenheim apricots (*Prunus armeniaca*), approximately twenty years old, and young French prunes (*Prunus domestica*) four years old. At Delhi, Muir peaches (*Prunus persica*) and French prunes were used during their fourth and fifth season in the orchard. At Davis, Muir peaches sixteen years old and French prunes of various ages were used.

MOISTURE DETERMINATIONS OF TREE TISSUES

During a part of the investigation, extensive studies were made on the moisture content of various parts of the tree. Before starting the investigation of the moisture content of the various tissues of the tree, extensive trial determinations were made, using large numbers of samples to determine the degree of reliability of the results obtained when using the methods described below. The samples were taken at three-hour intervals beginning at 6 A.M. Leaves from current growth of the season (shoots) in the upper fully exposed portion of the tree were quickly stripped off, and placed in the tin cans fitted with tight covers. All the leaves on the terminal foot of growth, except the terminal four or five, were used, without stopping to make an accurate count of the number. As the shoots used for stripping were chosen for uniformity in size and length, the number and weight of leaves secured by this method were approximately the same for all samples. Next, the terminal six inches of growth was removed. Buds and remaining leaves were carefully removed. Then the bark was stripped from the xylem, wiped dry with a towel, and wood and bark were placed in separate weighing bottles. In the same manner samples were taken of the basal six inches of growth. Two shoots were used for each sample and the samples were taken in duplicate.

Samples of trunk bark and trunk wood, and of root bark and root wood were taken with a carpenter's brace and auger bit. For the bark a 1-inch bit was used and for the wood a ¾-inch bit. The wood was taken to a depth of one-half inch. Uniform depth of boring was secured by boring to a file mark on the spiral of the auger. The chips were allowed to fall to a cloth spread on the ground and were then picked up as quickly as possible and placed in weighing bottles. Root samples were taken in the same way, using the part of the tree about eight inches below the surface of the soil just above the point where the main roots started to leave the main cylinder of the tree. This point was chosen for root samples in order to secure bark of approximately uniform thickness. Duplicate samples were not used in the trunk and root determination in order to avoid permanently injuring the trees by boring a large number of holes comparatively close together. The trunk samples were taken in a spiral beginning at the lower branches and ending four to six inches above the surface of the ground. The first samples were taken from the northeast side of the tree. The next samples were taken from the east or southeast and so on. By moving the point from which samples were taken for successive samples in a clockwise direction all samples could be secured from exposed portions of the tree without interference from holes previously bored in the trunk or roots. All samples were dried in a ventilated oven for forty-eight hours at 95°–100°C. The percentage of moisture was calculated on the dry weight of the material.

Samples were taken from both irrigated and non-irrigated trees at Davis and at Delhi during August and September, 1925. Four sets of samples were taken at weekly intervals at each place, the first at Davis on August 6, the first at Delhi on September 11, 1925. Thus, eight different pairs of trees were studied during the season. The experiment was carried on during the latter part of the summer because of the desirability of having the soil moisture on the non-irrigated plots reduced to a minimum so as to afford a marked contrast to the trees in the irrigated plots. All trees had formed terminal buds when the samples were taken. The trees which were adequately supplied with water are hereinafter referred to as "trees in moist soil"; the others, as "trees in dry soil." The moist soil plots were not allowed to reach the wilting coefficient during the experiment. The dry soil plots were allowed to remain at or below the wilting coefficient during the experiment. At other times the treatment given to the dry soil plots was consistent with good orchard practice.

EXPERIMENTS TO DETERMINE THE WIDTH OF STOMATAL OPENING

Experiments to determine the behavior of stomata on fruit trees were carried on at Mountain View and at Delhi during the summer of 1924. The leaf samples were taken in the manner previously described. During the early part of the season, there was but little difference in the degree of opening between stomata on the trees in moist soil and those on the trees in dry soil. In many cases the curves showing the amount of opening on the two trees were nearly parallel throughout the day.

On June 3, 1924, stomata on an apricot tree which had been irrigated a few days before behaved in an almost identical manner as with that of the stomata on a similar tree which had not been irrigated. Investigation showed that the soil around the apricot tree which had not been irrigated still contained available moisture, and under the comparatively mild climatic conditions which existed on that day, the stomata were able to open as wide as those on the tree which had been watered. The same results were obtained with peaches at Delhi on June 18, 1924, when soil samples showed that both the irrigated and the non-irrigated trees were still supplied with available moisture.

Still further evidence of this behavior was observed with French prunes and Muir peaches under the hotter and drier climatic conditions at Davis on July 9, 1925. The soil in the irrigated plot upon being sampled was found to contain but a small amount of water more than the non-irrigated plot on the day when the stomata samples were taken. Both soils were above the hygroscopic coefficient. The curves for the stomata from the trees in moist soil and the trees in dry soil show approximately the same characteristics. The results are shown in figures 1 and 2 and the data are given in table 1.

Numerous other trials both at Mountain View and at Davis with prune, apricot, and peach trees gave practically the same results as those just described. There was little or no difference in the stomatal behavior between the trees in the moist soil plots and those in the dry soil plots as long as the moisture content of both plots was above the hygroscopic point. In other words, decisive differences in stomatal behavior between trees in moist soil and those in dry soil were not obtained until after the latter trees had used up the available moisture in the root zone.

As the season advanced, differences in percentage of soil moisture between the plots kept well supplied with water and those which were not irrigated during the period of the experiment increased. These differences were reflected in the widths of the stomatal openings on the leaves of the respective trees. When the soil moisture in the plots with dry soil reached the point where water was not easily available to the tree, the stomata failed to open as wide as those on trees well supplied with moisture. Furthermore, the stomata on the trees in the dry plots often began to close at an earlier hour.

TABLE 1.

BEHAVIOR OF STOMATA ON FRENCH PRUNE AND ON MUIR PEACH TREES AT DAVIS, CALIFORNIA. July 9, 1925.

Time	Temperature °F.	Relative humidity per cent	Size of stomata in microns French prune				Size of stomata in microns Muir peach			
			Moist soil tree		Dry soil tree		Moist soil tree		Dry soil tree	
			Length	Width	Length	Width	Length	Width	Length	Width
5 a.m......	54	99	15.1	1.4	15.1	1.3	15.5	1.6	15.5	1.6
6 a.m......	57	98	15.5	1.7	15.5	1.6	15.9	1.9	15.5	2.5
7 a.m.......	61	89	14.3	2.3	15.9	1.6	15.1	3.3	15.9	2.9
8 a.m.......	67	80	15.9	2.7	15.9	2.6	16.8	3.8	16.6	3.8
9 a.m.......	73	69	15.9	2.6	15.1	2.7	15.9	4.2	15.5	3.8
10 a.m.....	82	52	15.1	2.9	15.1	2.5	16.4	4.1	14.7	3.0
11 a.m.....	87	46	16.1	3.1	15.5	2.7	15.5	3.0	15.5	2.6
12 a.m.....	88	45	15.1	3.2	15.1	3.2	15.5	2.4	15.9	3.1
1 p.m......	89	41	16.1	2.6	15.5	2.3	15.5	2.2	15.1	1.9
2 p.m.......	92	37	16.4	2.5	15.1	2.3	16.4	2.3	15.4	1.8
3 p.m.......	94	35	15.1	1.8	15.5	1.6	16.4	2.2	15.1	1.8
4 p.m.......	94	35	15.1	1.3	15.1	1.5	16.4	1.8	15.1	1.8
5 p.m.......	91	39	15.9	1.7	15.5	1.3	15.5	1.6	15.9	2.2
6 p.m.......	83	49	15.5	1.0	15.9	.8	15.5	1.7	14.3	2.0
7 p.m.......	73	63	15.5	1.0	15.9	1.0	15.9	1.4	15.5	1.8
8:30 p.m..	65	82	15.1	1.0	15.9	.8	15.9	1.3	14.3	1.1

NOTE:	Per cent
Percentage of moisture in soil around moist soil prune tree, 0–3 feet...........	–16.1
Percentage of moisture in soil around moist soil prune tree, 3–6 feet...........	–13.5
Percentage of moisture in soil around dry soil prune tree, 0–3 feet...............	–14.8
Percentage of moisture in soil around dry soil prune tree, 3–6 feet...............	–14.3
Percentage of moisture in soil around moist soil peach tree, 0–3 feet...........	–18.6
Percentage of moisture in soil around moist soil peach tree, 3–6 feet...........	–18.5
Percentage of moisture in soil around dry soil peach tree, 0–3 feet...............	–12.8
Percentage of moisture in soil around dry soil peach tree, 3–6 feet...............	–13.4
Calculated moisture equivalent, prune plot, 0–3 feet.......................................	–21.2
Calculated moisture equivalent, prune plot, 3–6 feet.......................................	–16.9
Calculated wilting coefficient, prune plot, 0–3 feet..	–11.5
Calculated wilting coefficient, prune plot, 3–6 feet..	– 9.2
Calculated hygroscopic coefficient, prune plot, 0–3 feet..................................	– 7.8
Calculated hygroscopic coefficient, prune plot, 3–6 feet..................................	– 6.3
Calculated moisture equivalent, peach plots, 0–3 feet.....................................	–18.6
Calculared moisture equivalent, peach plots, 3–6 feet.....................................	–16.4
Calculated wilting coefficient, peach plots, 0–3 feet..	–10.1
Calculated wilting coefficient, peach plots, 3–6 feet..	– 8.9
Calculated hygroscopic coefficient, peach plots, 0–3 feet................................	– 6.8
Calculated hygroscopic coefficient, peach plots, 3–6 feet................................	– 6.0

Results similar to those described in the preceding paragraph were obtained on many different dates during 1924, both at Delhi and at Mountain View. The curves given show the typical stomatal behavior for French prunes, August 5, 1924, at Mountain View (fig. 3); for Blenheim apricots, August 21, 1924, at Mountain View (fig. 4); and for Muir peaches October 1, 1924, at Delhi (fig. 5). A "high fog" at Mountain View on the morning of August 5 which persisted until about 9 A.M. may help to account for the fact that the stomata on the trees in the dry soil plots opened nearly as wide as those on the watered trees. However, as soon as the sun dispelled the fog, the relative humidity of the air was reduced, transpiration of the leaves probably increased, and the stomata began to close rapidly. The data for the stomatal measurements on French prunes at Mountain View, August 5, 1924, are given in table 2; for Blenheim apricots at

TABLE 2.

BEHAVIOR OF STOMATA ON FRENCH PRUNE TREES AT MOUNTAIN VIEW, CALIFORNIA. August 5, 1924.

Time	Temperature °F.	Relative humidity per cent	Size of stomata in microns			
			Moist soil tree		Dry soil tree	
			Length	Width	Length	Width
6 a.m.	54	99	13.9	1.6	12.6	1.2
7 a.m.	54	99	15.1	1.5	13.0	1.4
8 a.m.	58	99	14.6	1.9	15.5	1.5
9 a.m.	61	91	15.9	2.2	....	....
10 a.m.	68	81	14.6	2.4	15.5	2.1
11 a.m.	70	77	14.6	1.9	14.6	2.4
12 a.m.	73	75	17.2	3.0	14.1	2.2
1 p.m.	73	73	15.9	2.1	....	....
2 p.m.	75	70	15.5	2.3	....	....
3 p.m.	73	72	16.4	2.2	12.6	1.0
4 p.m.	71	75	16.4	2.1	14.3	1.1
5 p.m.	69	75	15.5	1.6	13.4	0.7
6 p.m.	65	84	14.6	1.9	14.3	0.7
7 p.m.	61	91	15.1	2.6	15.4	0.5
8:30 p.m.	58	98	15.9	1.4	15.9	0.0

NOTE:	Per cent
Percentage of moisture in soil, moist soil tree, 0–3 feet	−19.2
Percentage of moisture in soil, moist soil tree, 3–6 feet	−14.1
Percentage of moisture in soil, dry soil tree, 0–3 feet	−10.1
Calculated moisture equivalent	−22.0
Calculated wilting coefficient	−11.9
Calculated hygroscopic coefficient	−8.05

Mountain View, August 21, 1924, in table 3; and for Muir peaches at Delhi in table 4.* Stomatal measurements from an irrigated French prune at Delhi on the same day are given for purposes of comparison with the stomatal measurements of the peach. The similarity of curves for the peach and the prune when both trees were amply supplied with water is typical of what was found throughout the season. Evidently there is no great difference in the behavior of stomata of these two species.

TABLE 3.

BEHAVIOR OF STOMATA ON BLENHEIM APRICOTS AT MOUNTAIN VIEW, CALIFORNIA. August 21, 1924.

Time	Temperature °F.	Relative humidity per cent	Size of stomata in microns			
			Moist soil tree		Dry soil tree	
			Length	Width	Length	Width
9 a.m.	62	85	16.4	2.0	17.2	3.0
10 a.m.	65	82	18.1	2.8	17.0	3.0
11 a.m.	69	78	18.5	3.6	17.2	3.2
12 a.m.	70	77	17.3	3.6	17.6	2.5
1 p.m.	72	77	18.9	4.2	17.0	2.5
2 p.m.	71	78	19.7	4.4	17.0	2.3
3 p.m.	71	78	17.2	3.6	18.4	2.0
4 p.m.	70	81	17.2	2.5	18.5	1.9
5 p.m.	68	85	17.2	2.5	17.0	1.9
6 p.m.	64	92	17.6	2.9	18.1	2.0
7 p.m.	61	95	17.6	1.9	17.6	2.4
9 p.m.	57	99	18.4	1.2	18.1	1.6

NOTE:	Per cent
Percentage of moisture in soil, moist soil tree, 0–3 feet	–17.6
Percentage of moisture in soil, dry soil tree, 0–3 feet	–10.3
Calculated moisture equivalent	–22.0
Calculated wilting coefficient	–11.9
Calculated hygroscopic coefficient	–8.05

Twenty-four Hour Observations on Prune and Apricot Trees.—On July 22, 1924, stomata were measured on prune and apricot trees at Mountain View at hourly intervals throughout a twenty-four hour period beginning at 6 A.M. and ending at 5 A.M. the following morning. No extreme climatic conditions were encountered during the time the samples were being taken. The temperature ranged from a minimum of 55° F. at 6 A.M. to a maximum of 73° F. at 1 P.M. The relative humidity ranged from 98 per cent during the night down to 68 per cent at 1 P.M. In the morning a "high fog" persisted until about 10 A.M. This condition caused a comparatively wide degree of stomatal opening on the leaves of the trees in both plots. The stomata on the apricots in the moist soil plots reached a maximum width of 3.5 microns at 9 A.M. and the stomata on the trees in the dry soil plots

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* The second three feet of soil in the irrigated plot, because of the existence of a compacted layer about five feet beneath the surface, shows higher moisture equivalents and calculated wilting coefficients and hygroscopic points than the top three feet.

TABLE 4.

BEHAVIOR OF STOMATA ON MUIR PEACHES AND FRENCH PRUNES AT DELHI, CALIFORNIA. October 1, 1924.

Time	Temperature °F.	Relative humidity per cent	Size of stomata in microns					
			Moist soil peach tree		Dry soil peach tree		Moist soil prune tree	
			Length	Width	Length	Width	Length	Width
7 a.m.	55	94	14.3	1.7	15.1	2.1	14.2	3.6
8 a.m.	65	91	15.1	2.5	15.3	2.1	13.0	3.8
9 a.m.	71	78	14.2	2.5	15.3	2.3	12.1	3.8
10 a.m.	74	70	14.2	3.6	15.1	1.9	15.1	4.2
11 a.m.	76	67	14.2	3.3	15.3	2.0	14.2	4.5
12 a.m.	79	60	14.3	3.4	16 1	1.9	14.2	4.2
1 p.m.	82	57	14.3	3.3	16 3	1.9	....	....
2 p.m.	82	55	15.9	2.9	16.3	1.7	15.5	3.2
3 p.m.	82	51	14.3	2.9	15.1	1.5	17.2	1.7
4 p.m.	84	45	15.5	2.3	15.4	1.5	15.5	2.2
5 p.m.	81	48	15.9	1.9	15.9	1.4	15.5	2.0
6 p.m.	75	56	16.4	1.6	14.7	1.2	15.1	1.8
7 p.m.	73	61	16.8	0.9	16.8	1.1	15.5	1.6

NOTE:	Per cent
Percentage of soil moisture, moist soil tree, 0–3 feet	– 6.9
Percentage of soil moisture, moist soil tree, 3–6 feet	–12.9
Percentage of soil moisture, dry soil tree, 0–3 feet	– 1.2
Percentage of soil moisture, dry soil tree, 3–6 feet	– 2.0
Calculated moisture equivalent, moist soil tree, 0–3 feet	– 6.9
Calculated moisture equivalent, moist soil tree, 3–6 feet	–13.3
Calculated moisture equivalent, dry soil tree	– 6.5
Calculated wilting coefficient, moist soil tree, 0–3 feet	– 3.8
Calculated wilting coefficient, moist soil tree, 3–6 feet	– 7.2
Calculated wilting coefficient, dry soil tree	– 3.5
Calculated hygroscopic point, moist soil tree, 0–3 feet	– 2.5
Calculated hygroscopic point, moist soil tree, 3–6 feet	– 4.9
Calculated hygroscopic point, dry soil tree	– 2.4

reached a maximum width of 3.2 microns at the same hour. Thereafter, throughout the day the stomata on the moist plot trees showed a markedly wider degree of opening than the trees in the dry soil plots. The greatest closure in both cases occurred at 11 P.M., after which hour the stomata on both trees began to open.

The stomata of the prune trees in the moist soil plots attained a maximum width of 3.2 microns at 10 A.M. and then slowly began to close. On the trees in the dry soil plot, the maximum opening which was not reached until 2 P.M., was only 2.2 microns. The greatest average closure of stomata on trees in both plots was reached at 9 P.M., after which the stomata began to open slowly as in the case of the apricot trees. With both the apricots and the prunes, there seemed to be a tendency for the stomata to open slightly at 5 P.M. or 6 P.M. before finally closing to the minimum a few hours later. The data were so similar to those of a second twenty-four hour period on September 11 that only the latter are given in this paper.

On September 11, 1924, the stomata on Blenheim apricot and French prune trees were again studied throughout a twenty-four hour period. The stomata of the trees in dry soil did not open so wide as those on the tree in moist soil and began to close earlier in the day. This difference, which was particularly marked in the case of the French prune, may have been due to the rather severe climatic conditions which prevailed. A maximum temperature of 91° F. was reached at 1 P.M., while the relative humidity was 21 per cent at the same hour. The stomata showed the greatest closure between 8 P.M. and 10 P.M. Although there was bright moonlight until 4:30 A.M., this condition did not seem to have any effect on the opening of the stomata, which opened in much the same way as on July 22. The results are shown graphically on figures 6 and 7. The data for all four trees are given in table 5.

Behavior of Stomata on Different Parts of the Tree.—An experiment was carried out on September 15, 1925, at Davis to determine whether any difference existed in the behavior of stomata on different parts of the trees on moist soil and on dry soil. Five-year-old Robe de Sergeant prunes (*Prunus domestica*) which had made an average new growth of four feet were used. Samples were taken from the terminal leaf, from the tenth leaf below the terminal, from the twentieth leaf below the terminal, and from leaves produced on fruit-spurs low down on the main branches of the tree. The results are shown graphically in figure 8. The stomata from the tree in dry soil did not show much variation in their behavior. They all opened to approx-

TABLE 5.

BEHAVIOR OF STOMATA ON BLENHEIM APRICOT AND FRENCH PRUNE TREES AT MOUNTAIN VIEW, CALIFORNIA. September 11, 1924.

Time	Temperature °F.	Relative humidity per cent	Size of stomata in microns							
			Moist soil apricot tree		Dry soil apricot tree		Moist soil prune tree		Dry soil prune tree	
			Length	Width	Length	Width	Length	Width	Length	Width
6 a.m.......	46	100	13.9	3.0	14.3	2.4	14 3	1.6	13.9	0.7
7 a.m.	47	94	16.8	3.2	15.9	2.2	15.5	1.5	13.9	1.1
8 a.m.......	62	77	16.4	3.5	16.4	2.7	15.1	2.1	15.5	1.5
9 a.m.......	72	63	17.2	3.8	16.8	3.1	15.5	2.5	16.4	1.7
10 a.m.....	81	47	15.1	3.9	19.0	3.6	15.1	4.3	15.1	1.7
11 a.m. ..	87	34	15.9	4.0	17.2	3.0	15.5	4 4	14.6	1.3
12 a.m....	93	22	15.5	3.9	16.4	3.4	13.9	3.8	14.6	1.1
1 p.m.......	95	21	16.8	3.4	14.6	2.9	16.4	2.6	13.9	0.8
2 p.m.......	94	21	16.4	2.8	16.4	2.9	13.9	3.0	13.9	1.0
3 p.m.......	93	21	17.6	2.5	19.0	2.5	15.1	2.6	13.9	1.5
4 p.m.......	91	28	15.5	2.7	16.4	2.2	14.3	2.0	15.1	1.6
5 p.m......	89	27	15 1	2.8	16.8	2.0	14.3	2.1	14.6	1.0
6 p.m......	74	75	18.5	2.4	15.5	1.9	15.5	1.8	13.0	1.3
7 p.m.......	69	84	18.1	1.8	15.9	2.0	15.1	1.7	14.6	1.0
8 p.m.......	65	85	18.1	2.6	17.2	2.2	14.3	1.6	14.6	0.2
9 p.m.......	61	89	16.8	0.5	16.8	1.6	15.5	0.7	16.4	0.2
10 p.m.....	58	92	15.9	0.7	16.4	1.0	15.5	0.9	14.6	0.4
11 p.m.....	56	92	16.4	1.0	15.5	1.7	14.3	1.0	15.1	0.4
12 p.m.....	54	92	16.4	1.4	16.4	1.8	15.5	0.6	....	....
1 a.m.......	51	94	18.5	2.1	18.5	2.2	15.1	1.4	15.5	0.4
2 a.m.......	50	95	16.8	2.1	16.8	2.3	15.5	1.5	14.6	0.7
3 a.m.......	50	95	18.1	2.5	16.4	2.1	14.6	1.3	14.6	0.8
4 a.m.......	48	96	17.6	2.3	15.5	2.1	15.1	1.5	15.1	0.6
5 a.m.......	49	99	16.8	2.5	16.1	2.1	14.6	1.7	15.5	0.6

NOTE:	Per cent
Percentage of moisture in soil, moist soil apricot tree, 0–3 feet......................	–15.0
Percentage of moisture in soil, dry soil apricot tree, 0–3 feet..........................	– 9.9
Percentage of moisture in soil, moist soil prune tree, 0–3 feet	–14.9
Percentage of moisture in soil, moist soil prune tree, 3–6 feet..........................	–10.5
Percentage of moisture in soil, dry soil prune tree, 0–3 feet	– 9.9
Calculated moisture equivalent...............	–22.0
Calculated wilting coefficient..	–11.9
Calculated hygroscopic coefficient..	–8.05

imately the same width (about 1 micron). The leaves from the branch on the tree in moist soil showed marked differences in amount of stomatal opening. The first and tenth leaves showed a stomatal opening of slightly more than 2 microns before they started to close. The stomata on the twentieth leaf opened to 3 microns and those on the spur leaf to 3.8 microns, before closing.

It is interesting to note how closely these results agree with those obtained by the author[9] in 1920, which showed that the terminal leaves on current season's shoots transpired less rapidly than the spur leaves further down on the main branches of the tree. The data are given in table 6.

Effect of Shade on Stomatal Behavior.—The effect of continuous shade on stomata was shown by an experiment carried on at Davis, July 28, 1925. Three Elberta peach trees were used. One tree in moist soil and one tree in dry soil under open orchard conditions, and in addition one tree growing in well moistened soil under the shade of a muslin tent, which was erected soon after growth started in the spring, were used. The shaded tree was covered with a tent stretched on a framework of sufficient size to allow normal growth of the tree. The cloth extended down on the sides of the tent to within three feet of the ground leaving an open space on all sides, which allowed free circulation of air. Before being enclosed in the tent, the shaded tree had been given the usual orchard treatment. The air temperature in the tent and in the shade of the tree in the open were practically the same throughout the day. The evaporation rate, which was determined with porous cup atmometers, within the tent was approximately two-thirds of that in the direct sunlight during the period from 8 A.M. July 28 to 8 A.M. July 29.

Leaves on the shaded tree were much larger but thinner than leaves on the trees growing in the open. The stomata of the leaves on the shaded tree as shown in figure 9 opened much later than did those on the trees outside of the tent. They opened wider than the stomata on the tree in dry soil outside of the tent, but not so wide as those on the unshaded tree in moist soil. After 1 P.M. the width of the stomatal opening on the leaves of the shaded tree and of the irrigated tree remained about the same. The stomata on the dry tree began to close earlier than either the irrigated or the shaded tree. Essentially similar results were obtained a week earlier when the percentage of soil moisture around both the irrigated and the shaded tree was much lower than in the case for which the curves are given (fig. 9). The data are given in table 7.

TABLE 6.

BEHAVIOR OF STOMATA ON ROBE DE SERGEANT PRUNE TREES AT DAVIS, CALIFORNIA. September 15, 1925.

Time	Temperature °F.	Relative humidity per cent	Size of stomata in microns							
			Terminal leaf				Tenth leaf			
			Moist soil tree		Dry soil tree		Moist soil tree		Dry soil tree	
			Length	Width	Length	Width	Length	Width	Length	Width
6 a.m.......	52	97	15.5	0.4	15.5	0.6	15.9	0.8	15.5	0.5
8 a.m.......	64	91	15.1	2.4	15.5	1 3	15.5	1.5	15.9	0.8
10 a.m.....	74	65	15.1	2.1	15.9	0.8	15.9	2.1	16 4	1.1
12 a.m.....	81	52	15.1	1.6	15.5	0 9	15.5	1.9	15.9	1.1
2 p.m.......	84	46	15.5	1.4	15.5	1.0	15.9	1.5	15.9	0.8
4 p.m.......	82	47	15.9	1.0	15.9	0.8	15.9	1.3	15.9	1.0
6 p.m.......	78	55	15.5	0.5	15.9	0.4	15.5	0.4	16.4	0.9
			Twentieth leaf				Spur leaf			
6 a.m.......	52	97	15.5	1.3	15.9	0.8	15.5	0.7	15.5	0.4
8 a.m.......	64	91	15.9	3.0	15.5	1.1	16.8	3.2	15.9	0.5
10 a.m....	74	65	15.5	2.2	15.5	1.0	15.9	3.8	15.9	0.8
12 a.m....	81	52	15.9	1.4	15.9	0.7	15.1	3.4	15.9	1.0
2 p.m.......	84	46	15.5	1.5	15.9	0.8	15.5	1.9	15.9	0.6
4 p.m.......	82	47	15.5	2.1	15.5	0.6	15.5	2.3	15.5	0.4
6 p.m.......	78	55	15.1	1.4	15.9	0.8	15.1	1.6	16.4	0.5

NOTE:	Per cent
Percentage of soil moisture, moist soil tree, 0–3 feet	—15.5
Percentage of soil moisture, moist soil tree, 3–6 feet	—17.6
Percentage of soil moisture, dry soil tree, 0–3 feet	— 8.1
Percentage of soil moisture, dry soil tree, 3–6 feet	— 8.9
Calculated moisture equivalent, 0–3 feet	—20.0
Calculated moisture equivalent, 3–6 feet	—25.2
Calculated wilting coefficient, 0–3 feet	—10.8
Calculated wilting coefficient, 3–6 feet	—13.7
Calculated hygroscopic coefficient, 0–3 feet	— 7.3
Calculated hygroscopic coefficient, 3–6 feet	— 9.3

TABLE 7.

BEHAVIOR OF STOMATA ON ELBERTA PEACH TREES AT DAVIS, CALIFORNIA. July 28, 1925.

Time	Temperature °F.	Relative humidity per cent	Size of stomata in microns					
			Moist soil tree		Dry soil tree		Shaded tree	
			Length	Width	Length	Width	Length	Width
5 a.m.	51	99	15.5	1.8	15.5	1.7	15.9	0.9
6 a.m.	52	99	16.9	1.9	15.9	1.8	15.5	1.0
7 a.m.	58	99	15.9	2.0	14.6	2.0	14.6	1.2
8 a.m.	63	92	15.9	2.0	15.5	1.9	15.1	1.6
9 a.m.	67	85	15.1	1.8	15.5	1.6	15.9	1.5
10 a.m.	76	69	15.5	2.2	15.5	1.6	15.5	1.8
11 a.m.	83	58	16.4	2.5	15.9	1.6	14.6	2.1
12 a.m.	87	46	15.9	2.0	16.1	1.3	15.5	1.9
1 p.m.	90	40	15.5	1.9	15.1	1.1	15.1	2.0
2 p.m.	94	36	15.5	1.7	15.1	1.0	15.9	1.7
3 p.m.	95	34	15.5	1.4	15.5	0.8	15.9	1.5
4 p.m.	96	35	15.9	1.3	15.5	0.8	15.9	1.4
5 p.m.	94	36	16.1	1.3	15.9	0.9	15.1	1.2
6 p.m.	93	40	15.1	1.1	16.4	0.7	14.6	0.9
7 p.m.	85	44	15.5	1.0	15.5	0.7	15.5	0.7
8 p.m.	75	52	15.5	0.9	....	....	16.1	0.4

NOTE:	Per cent
Percentage of soil moisture, moist soil tree, 0–3 feet	25.6
Percentage of soil moisture, moist soil tree, 3–6 feet	27.4
Percentage of soil moisture, dry soil tree, 0–3 feet	9.7
Percentage of soil moisture, dry soil tree, 3–6 feet	15.7
Percentage of soil moisture, shaded tree, 0–3 feet	24.5
Percentage of soil moisture, shaded tree, 3–6 feet	31.0
Calculated moisture equivalent, moist soil and shaded tree, 0–3 feet	22.4
Calculated moisture equivalent, moist soil and shaded tree, 3–6 feet	29.4
Calculated moisture equivalent, dry soil tree, 0–3 feet	29.5
Calculated moisture equivalent, dry soil tree, 3–6 feet	25.2
Calculated wilting coefficient, moist and shaded tree, 0–3 feet	12.2
Calculated wilting coefficient, moist and shaded tree, 3–6 feet	15.9
Calculated wilting coefficient, dry soil tree, 0–3 feet	16.0
Calculated wilting coefficient, dry soil tree, 3–6 feet	13.7
Calculated hygroscopic coefficient, moist and shaded tree, 0–3 feet	8.3
Calculated hygroscopic coefficient, moist and shaded tree, 3–6 feet	10.8
Calculated hygroscopic coefficient, dry soil tree, 0–3 feet	10.6
Calculated hygroscopic coefficient, dry soil tree, 3–6 feet	9.3

EXPERIMENTS ON THE DETERMINATION OF MOISTURE CONTENT OF VARIOUS PARTS OF THE TREE IN RELATION TO STOMATAL MOVEMENT

The data showing the moisture content of the leaves, twigs, trunk, and roots of the trees studied are shown in figures 10 to 18. Because of the difficulties of showing the data concerning the stomatal movement, temperature, and relative humidity on the same charts with the percentage of moisture in the various tissues, these data are shown in figure 19. Data for the determination made on September 4, 1925, at Davis, which are typical of all the results obtained, are given in table 8. The data for the other seven weeks of the experiment are on file in the office of the Pomology Division of the University of California. A summary of the soil moisture conditions at Davis and at Delhi is given in table 9.

TABLE 8.

MOISTURE CONTENT IN PERCENTAGE OF DRY WEIGHT OF LEAVES, TWIGS, TRUNK, AND ROOTS OF TREES IN MOIST AND IN DRY SOIL AT DAVIS.
September 4, 1925.

Time	Leaves		Terminal twig bark		Terminal twig wood		Basal twig bark	
	Moist soil	Dry soil	Moist soil	Dry soil	Moist soil	Dry soil	Moist soil	Dry soil
6 a.m.	164.8	151.3	162.8	147.8	118.9	103 4	142.0	129.3
9 a.m.	149.5	141.5	153.3	125.3	107.6	80.9	138.5	116.1
12 m.	146.1	132.6	146.4	123.0	107.3	84.5	129.4	110.9
3 p.m.	142.4	135.3	139.4	121.1	90.6	85.5	135.3	105.8
6 p.m.	143.0	133.9	149.0	131.3	101.2	92.7	135.7	110.0

Time	Basal twig wood		Trunk bark		Trunk wood		Root bark		Root wood	
	Moist soil	Dry soil	Moist soil	Dry soil	Moist soil	Dry soil	Moist soil	Dry soil	Moist soil	Dry soil
6 a.m.	72.0	63.2	85.2	67.4	65.3	54.0	126.9	100.0	74.1	63.1
9 a.m.	61.2	58.3	69.9	68.0	52.2	49.2	116.2	84.1	63.7	53.4
12 m.	56.7	57.6	72.7	64.5	53.8	48.1	111.5	90 3	71.7	54.1
3 p.m.	57.0	56.4	69.4	65.5	50.9	48.7	106.0	86.9	62.6	58.7
6 p.m.	65.9	60.3	86.1	66.9	56.2	50.2	113.8	96.5	65.1	55.5

Percentages of moisture calculated on the dry weight of the material are plotted against time. The curves in the upper part of

TABLE 9—SUMMARY OF SOIL MOISTURE CONDITIONS EXPRESSED AS PERCENTAGE OF DRY WEIGHT OF TREES AT DAVIS AND DELHI FROM WHICH WATER DEFICIT DATA WERE OBTAINED.

Date	Location	Tree in moist soil								Tree in dry soil							
		First three feet				Second three feet				First three feet				Second three feet			
		Per cent moisture	Moisture equivalent	Wilting coefficient	Hygroscopic coefficient	Per cent moisture	Moisture equivalent	Wilting coefficient	Hygroscopic coefficient	Per cent moisture	Moisture equivalent	Wilting coefficient	Hygroscopic coefficient	Per cent moisture	Moisture equivalent	Wilting coefficient	Hygroscopic coefficient
8-6-25	Davis	14.5	16.7	9.1	6.2	11.8	14.5	7.8	5.3	9.7	18.6	10.1	6.8	9.5	16.4	8.9	6.0
8-13-25	Davis	13.7	15.2	8.3	5.6	10.5	12.6	6.8	4.6	9.8	18.6	10.1	6.8	10.4	16.4	8.9	6.0
8-25-25	Davis	14.8	18.8	10.2	6.9	8.5	16.2	8.8	5.9	9.3	18.6	10.1	6.8	10.1	16.4	8.9	6.0
9-4-25	Davis	17.9	18.8	10.2	6.9	15.1	16.2	8.8	5.9	8.6	18.6	10.1	6.8	7.1	16.4	8.9	6.0
9-11-25	Delhi*	13.6	5.8	3.2	2.1	21.5	13.8	7.5	5.0	1.7	6.5	3.5	2.4	2.6	6.5	3.5	2.4
9-18-25	Delhi*	6.5	5.8	3.2	2.1	15.2	13.8	7.5	5.0	1.3	6.5	3.5	2.4	1.7	6.5	3.5	2.4
9-25-25	Delhi*	9.4	5.8	3.2	2.1	17.4	13.8	7.5	5.0	1.5	6.5	3.5	2.4	2.5	6.5	3.5	2.4
10-2-25	Delhi*	3.0	5.8	3.2	2.1	14.0	13.8	7.5	5.0	1.5	6.5	3.5	2.4	2.9	6.5	3.5	2.4

* Abnormally high moisture content of soil at Delhi was due to a break in irrigating pipe line and impervious layer of soil which did not permit water to drain away to lower levels.

the chart are for the results obtained at Davis; in the lower, at Delhi. In each case the results from the tree on moist soil are shown on the left and the tree in dry soil on the right. The dates show the time of taking the samples.

Various irregularities in the curves occurred. Some of these were due to errors of sampling, inevitable with the type of material used. A few of the irregularities were due to other causes. Thus, some samples of leaves late in the season showed an abnormally high percentage of moisture at 6 A.M. This particular fact can undoubtedly be attributed to the dew which could not be removed from the leaves satisfactorily. On September 11 at Delhi, cloudy weather persisted until about 8:30 A.M. As a result, the leaves and terminal twigs of both trees in moist soil and trees in dry soil did not show a decrease in moisture content until after 9 A.M. On August 25 at Davis it was necessary to use some rather small roots for the determination of moisture in the root bark. As a consequence the curve for the root bark of the tree in moist soil rose until 12 o'clock instead of falling as was usually the case.

The similarity of the curves, particularly for the leaves and twigs, was apparent. There was a decrease in moisture content for the various tissues from 6 A.M. to 9 A.M. or from 6 A.M. to 12 noon, and there was an increase in moisture content from 3 P.M. to 6 P.M. The significance of these differences was tested by Student's Method[19] and, if it is assumed that this method is applicable to this case, the differences are significant. In a few cases where the significance of the differences between 6 A.M. and 9 A.M. was indicated by rather short odds, when the differences for the same tissues were calculated from 6 A.M. to 12 noon much greater odds were obtained.

DISCUSSION OF RESULTS OBTAINED IN THE DETERMINATION OF MOISTURE CONTENT OF VARIOUS TREE TISSUES

The curves showing the behavior of the stomata on the trees in moist soil and in dry soil during the season of 1925, as shown in figure 19, are essentially similar to, and show the same characteristics which were shown in, the detailed studies on stomatal movement in 1924. Generally speaking, the stomata showed a rather uniform behavior. They began to close as a rule between 9 A.M. and 12 noon. The stomata on the trees in dry soil consistently showed less opening than did those on the trees in moist soil. These curves are given in a separate figure because they could not be satisfactorily grouped on the charts showing the moisture content of the various tree tissues studied.

A complete summary of the soil moisture conditions for the top three feet and the second three feet of soil are given in table 9. The moisture equivalents, wilting coefficients, and hygroscopic coefficients expressed as percentage on a dry weight basis are given for purposes of comparison. The trees used in the experiment were growing in similar types of soil, but were handled in such a way as to give extreme conditions of soil moisture.

As may be seen from the table, the trees in moist soil were abundantly supplied with moisture, while those in dry soil usually had little or no available moisture to draw upon. The decisive results obtained may have been due to the fact that there was such a marked difference in soil moisture content between the plots in the experiment.

One of the most striking features of the moisture curves, as shown in the accompanying figures, was their similarity. In general, there was a decrease in moisture content of all parts studied between 6 A.M. and 9 A.M., and an increase in moisture content between 3 P.M. and 6 P.M. These results were similar to those obtained by Livingston and Brown,[13] who found that the minimum water content of certain desert plants occurred between 1 P.M. and 5 P.M. and then rose to a maximum at 7 P.M. One exception to this rule occurred on September 11 at Delhi, when the leaves, bark, and wood of the terminal ends of twigs failed to show a decrease until after 9 o'clock. This exception can probably be explained by the fact that the weather remained cloudy until after 8:30 A.M. The fall and rise of the water content of all the measured portions seemed to be associated with, or at least occurred at practically the same time as the opening and closing of the stomata.

The succulent portions of the tree, i.e., the leaves, and the terminal and basal portions of the twigs, showed a marked progressive decrease in water content during the early part of the season, which meant that these portions were increasing in dry matter. This decrease in water content of the leaves and twigs was slight after the middle of September. Trees in moist soil both at Davis and at Delhi showed a relatively greater decrease in water content from week to week than did the trees in dry soil. The trunks and roots did not show such a marked decrease in water content from week to week as the season advanced, although the decrease was fairly noticeable during the first few weeks of the experiment.

The leaves and terminal twigs of the trees in moist soil showed a consistently higher water content than these parts from the trees in dry soil. The same condition seemed to hold true for certain series of samples from the older tissues of the trees. At Delhi the wood and bark of trunks and root of trees growing in moist soil contained more water than the wood and bark of trees growing in dry soil. This difference seemed remarkable when the method of sampling was considered. With the older portions of the tree, a considerable part of each sample consisted of old and probably inactive tissue. This old tissue increased the relative amount of dry matter and may have tended to mask the results. It is also interesting to note that with the wood of the basal part of the twig, there was no great difference between the trees grown in moist soil and those grown in dry soil.

The comparatively high moisture content of the root bark on the trees in moist soil can probably be accounted for by the fact that these trees were irrigated two or three days before the sample was taken. The outer layers of bark may have absorbed and held sufficient water to account for this difference. Also, there was practically no loss by evaporation from the surface of the bark. The difference in water content between the trunk bark and the root bark may also be explained in the same way.

In general, the trunk and root samples, particularly in the case of the bark, showed greater irregularity than did the samples of leaves and twigs. This irregularity may have been due to the fact that old outer bark does not slough off evenly. There was no satisfactory method of judging the thickness of bark at the point chosen for taking samples. Furthermore, the wood samples sometimes showed evidence of brown tissue, the presence of which could not be foretold before making the boring. These factors which could not be guarded against probably contributed to the irregularity in the results obtained.

The bark of the terminal and basal portions of the twigs contained a larger percentage of moisture than the wood of these parts, except for the succulent terminals early in the season. This fact might suggest the relatively more rapid increase of dry matter in the xylem than in the phloem. Essentially the same condition was observed in the bark and wood of the older portions of the tree, but the lower moisture content of the wood in this case may have been due to depth of boring, as mentioned in a preceding paragraph.

Succulent portions of the trees during the latter part of the season seemed to resist loss of water beyond a certain point. Thus, the leaves on the trees in moist soil at Delhi, during the last three weeks of the experiment reached approximately a common minimum during the middle of the day. The same thing occurred in the case of the terminal twig portions of the trees at Delhi in both the moist and the dry soils.

It was evident that more water could not have been removed from these tissues under the given climatic and soil moisture conditions. In other words, the twigs had reached a certain stage of maturity where they resisted loss of moisture below a certain point. Inasmuch as this stage of maturity was reached at approximately the same time for the trees both in the well irrigated soil and in the dry soil, it seems evident that the data presented in this paper have an important bearing on a number of questions regarding the relation between irrigation and the hardening or maturing of the wood and buds of peach trees. It also seems evident from these results that the single factor of high soil-moisture content is insufficient to account for various types of so-called "winter injury," where these troubles occur, particularly if the injury seems to be influenced by the immaturity of the new growth of the tree.

The results presented in this paper indicate a relationship between stomatal movement and water content of various tissues of peach trees in California, and further, show that this relationship is markedly influenced by whether moisture is available in the soil or not. The general opening of stomata during the early morning hours is rapidly followed by a decrease in the water content of the bark and wood of the tree. The leaves and succulent twigs show a relatively great loss early in the day, and this fluctuation is rapidly propagated back to the roots. It may be detected in the root tissues as early as 9 A.M. Later in the day when the stomata begin to close, there is an increase in moisture content in all parts of the trees. The loss which takes place during the morning and early afternoon is rapidly replenished between 3 P.M. and 6 P.M.

While no data were secured during the progress of this particular experiment upon the actual measuring of transpiration, some were obtained on this phase of the question in 1920 while using many of the same trees included in the 1924 and 1925 experiments. A study of the original notes obtained in 1920 indicates that under similar conditions of climate and soil moisture, the curves for stomatal opening and for transpiration are parallel. Edith Shreve[18] has shown that a similar relation holds for leaves of *Parkinsonia microphylla*. Transpiration, however, in practically every case began to decrease somewhat before the average time for the beginning of stomatal closure. Thus, the present data in connection with those published in 1920 indicate a relationship between stomatal movement, transpiration and moisture content of the various tissues of peach trees.

The rapid loss of moisture which accompanies stomatal opening may help to explain why stomata, in many cases, begin to close before the light has reached its greatest intensity. Under conditions favorable for maintaining turgidity such as occur during the night, the stomata are sensitive to the action of light and probably are controlled chiefly by it. Thus, with the coming of daylight, the stomata open rapidly. Next, water loss occurs until such time as the guard cells or surrounding epidermal cells begin to lose their turgidity. When the cells adjacent to the stomata or the guard cells, themselves, are no longer turgid, closure of the stomata begins even if light conditions are favorable for them to remain open. Thus it seems that light is important in bringing about the opening of stomata when the leaves are turgid, but when the cells around the stomata have lost sufficient water to cause them to lose their turgidity and the guard cells themselves have lost a small amount of moisture, a factor opposing the influence of light is introduced which is sufficient to cause the stomata to begin to close.

The sensitive response to changes in stomatal opening or closing as shown by corresponding fluctuation in the water content of the tree tissues seems to furnish additional evidence in support of the cohesion theory[5] of the rise of water in trees. If the water in the conducting tissues of the plant is continuous, as claimed by the supporters of this theory, the effects of any water loss from the cells surrounding the stomatal cavity should be quickly noticeable in the adjoining cells and, furthermore, should be rapidly propagated down to the roots. Data from well watered trees and from trees in dry soil secured for eight successive weeks during the summer of 1925 show that diurnal fluctuations in water content of the leaves of peach trees are propagated down to the roots with remarkable speed.

SUMMARY

1. The stomata of peach, prune, and apricot trees reached their maximum degree of opening between 9 A.M. and 12 o'clock noon, after which they began to close. The greatest closure of stomata in prune and apricot trees was observed between 8 P.M. and 11 P.M.

2. Peach, prune, and apricot trees growing under conditions of little or no available soil moisture showed a smaller maximum stomatal opening than trees growing in soil containing a supply of available moisture.

3. Leaves at the apex of vigorous current season's shoots of prune trees growing in moist soil, showed less stomatal opening than older leaves farther back on the branch. On the prune trees which were suffering for water, all leaves on various parts of the tree showed approximately the same small degree of opening.

4. Stomata on shaded peach trees in moist soil did not reach the maximum degree of opening until several hours after those of trees in moist soil under open orchard conditions.

5. The decrease in moisture content which occurs in the leaves of peach trees, under California conditions, shortly after 6 A.M. is propagated backward rapidly and may be detected in all parts of the tree as early as 9 A.M. This loss of moisture is partly replenished between 3 P.M. and 6 P.M.

6. Decrease in moisture content of various tissues of peach trees was observed in many cases before the stomata had reached their maximum opening. Replacement of this loss began in the afternoon while the stomata were still open and while climatic conditions were still favorable for transpiration.

ACKNOWLEDGMENTS

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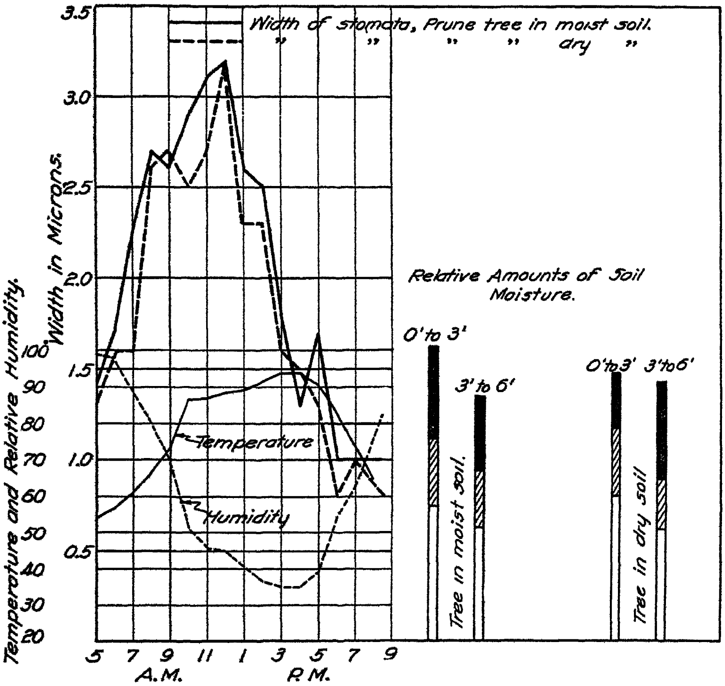

Fig. 1. Width of stomatal opening on prune trees in moist soil and in dry soil at Davis, California, July 9, 1925. Temperature and relative humidity are shown by light lines in lower left-hand corner. Relative amount of soil moisture above the wilting coefficient is shown by solid black column; relative amount of soil moisture below hygroscopic coefficient is shown by the unshaded portion.

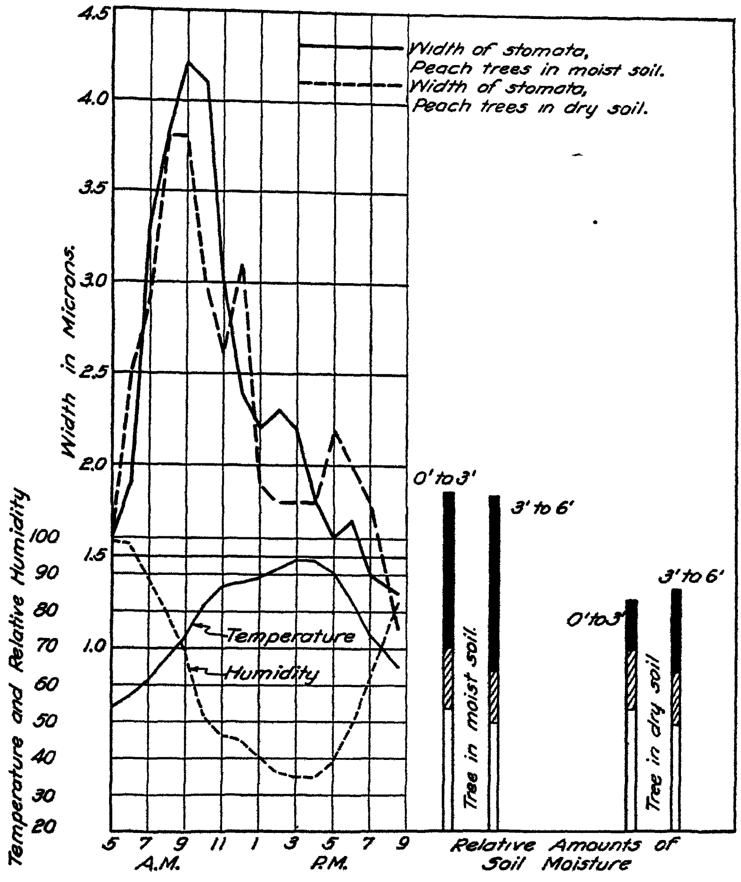

Fig. 2. Width of stomatal opening on peach trees in moist soil and in dry soil at Davis, California, July 9, 1925. Temperature and relative humidity are shown by light lines in lower left-hand corner. Relative amount of soil moisture above the wilting coefficient is shown by solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

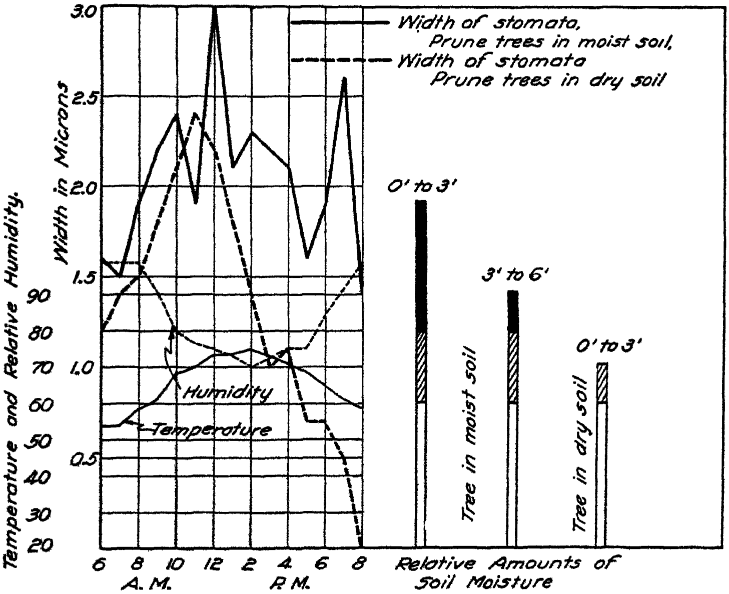

Fig. 3. Width of stomatal opening on prune trees in moist soil and in dry soil at Mountain View, California, August 5, 1924. Temperature and relative humidity are shown by light lines in the lower left-hand corner. Relative amount of soil moisture above wilting coefficient is shown by solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

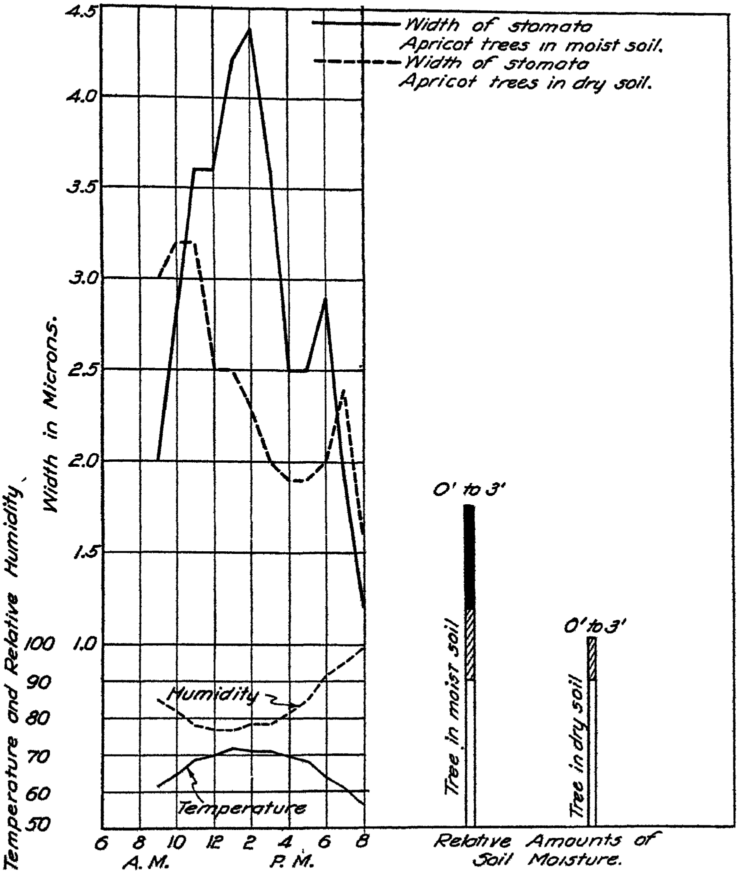

Fig. 4. Witdh of stomatal opening on apricot trees in moist soil and in dry soil at Mountain View, California, August 21, 1924. Temperature and relative humidity are shown in lower left corner. Relative amount of soil moisture above the wilting coefficient is shown by solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

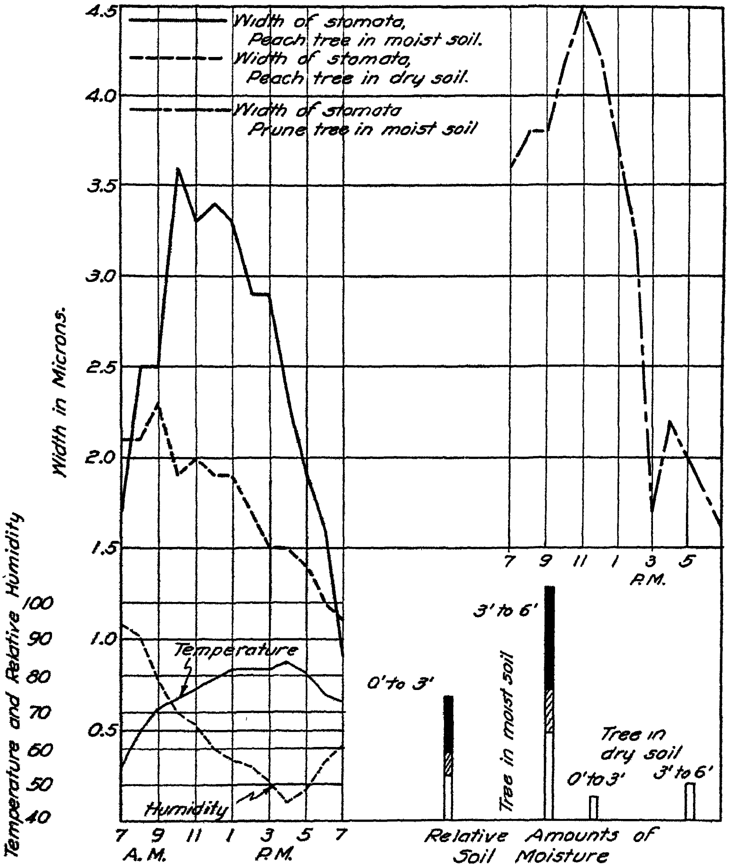

Fig. 5. Width of stomatal opening on peach and prune trees in moist soil and peach tree in dry soil at Delhi, October 1, 1924. Temperature and relative humidity are shown in lower left corner. Relative amount of soil moisture above wilting coefficient is shown by solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

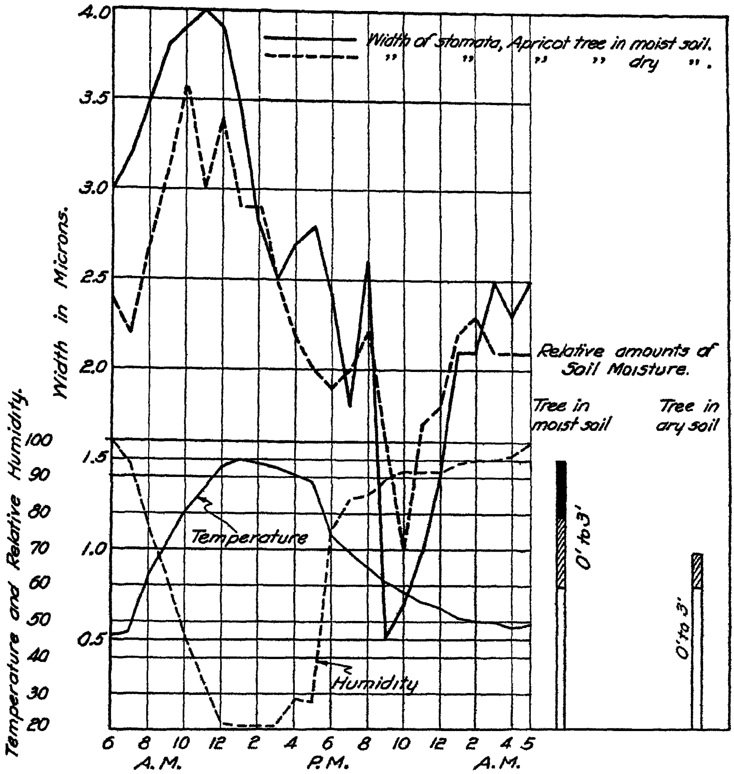

Fig. 6. Width of stomatal opening on apricot trees in moist soil and in dry soil at Mountain View, September 11, 1924. Temperature and relative humidity are shown in lower left corner. Relative amount of soil moisture above the wilting coefficient is shown by the solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

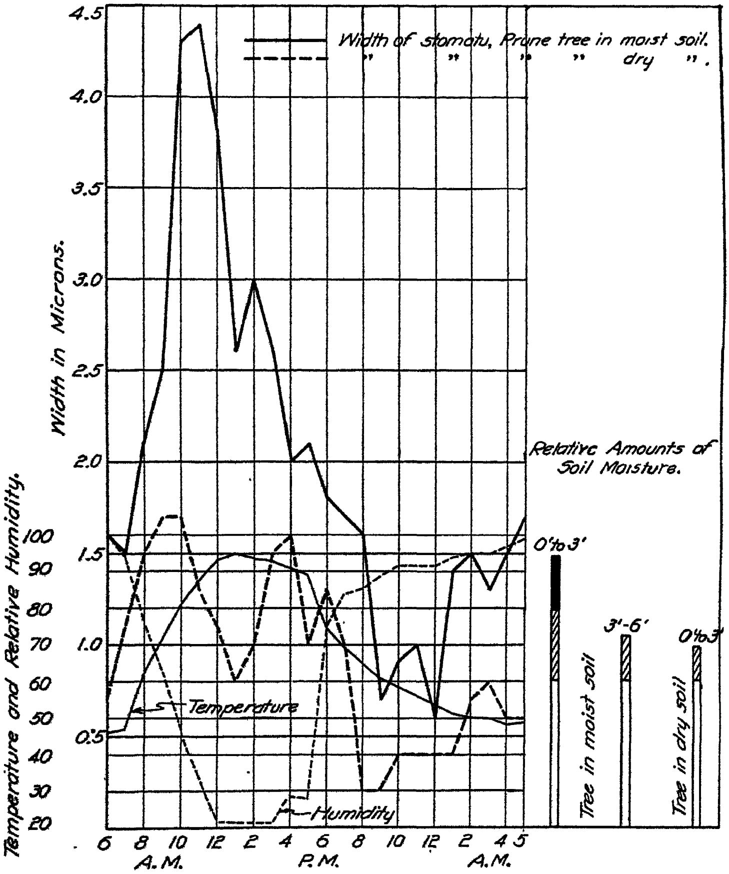

Fig. 7. Width of stomatal opening on prune trees in moist soil and in dry soil at Mountain View, California, September 11, 1924. Temperature and relative humidity are shown by the light lines in the lower left corner. Relative amount of soil moisture above the wilting coefficient is shown by the solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

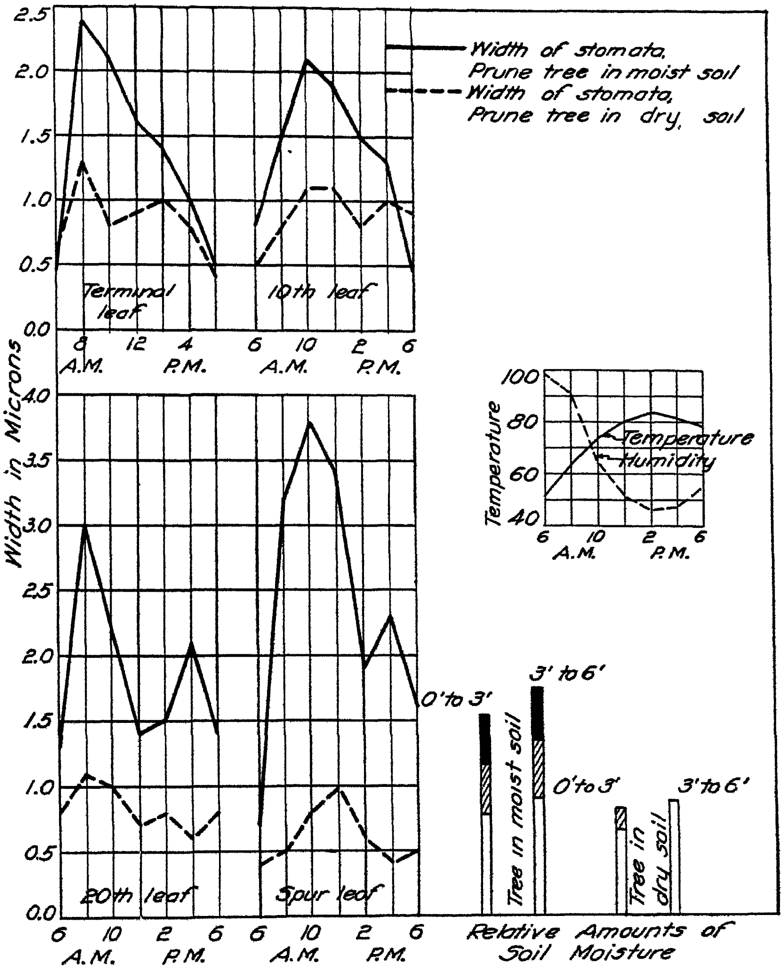

Fig. 8. Width of stomatal opening on leaves on different parts of strong shoots and on spurs found on prune trees in moist soil and in dry soil at Davis, California, September 15, 1925. Temperature and relative humidity are shown at the right. Relative amount of soil moisture above the wilting coefficient is shown by the solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

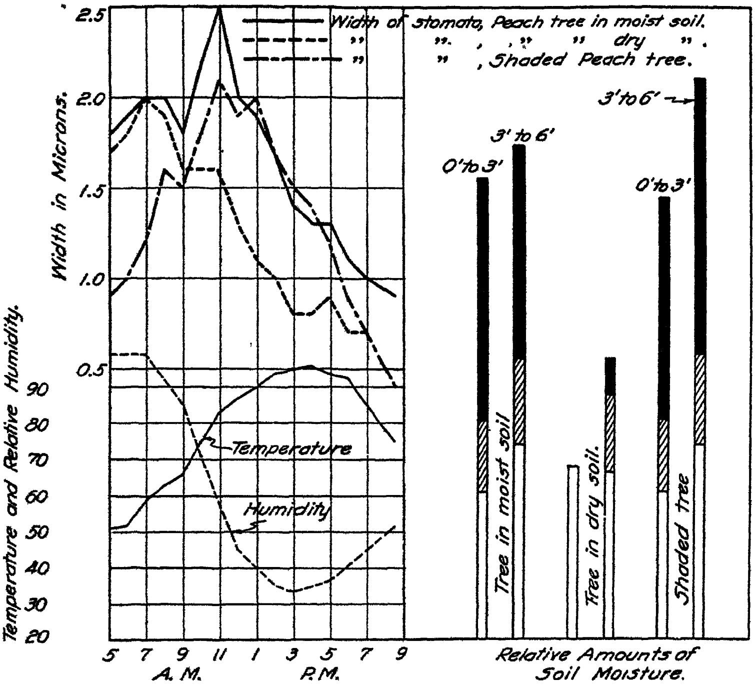

Fig. 9. Width of stomatal opening on shaded peach tree in moist soil and unshaded peach trees in moist soil and in dry soil at Davis, California, July 28, 1925. Temperature and relative humidity are shown in the lower left-hand corner. Relative amount of soil moisture above the wilting coefficient is shown by the solid black column; relative amount of soil moisture below the hygroscopic coefficient is shown by the unshaded portion.

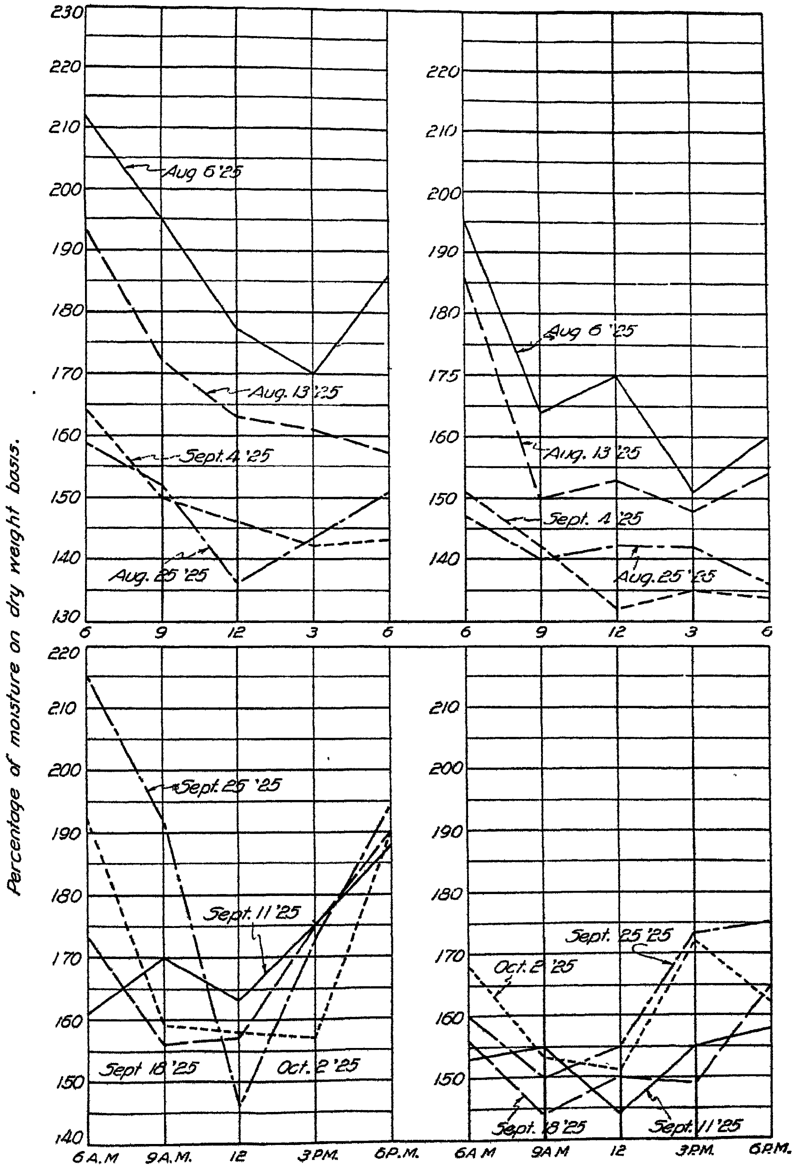

Fig. 10. Curves showing fluctuation in water content of peach leaves. Results from trees in moist soil shown on the left; from trees in dry soil, at the right. The upper curves show results obtained at Davis; the lower, at Delhi.

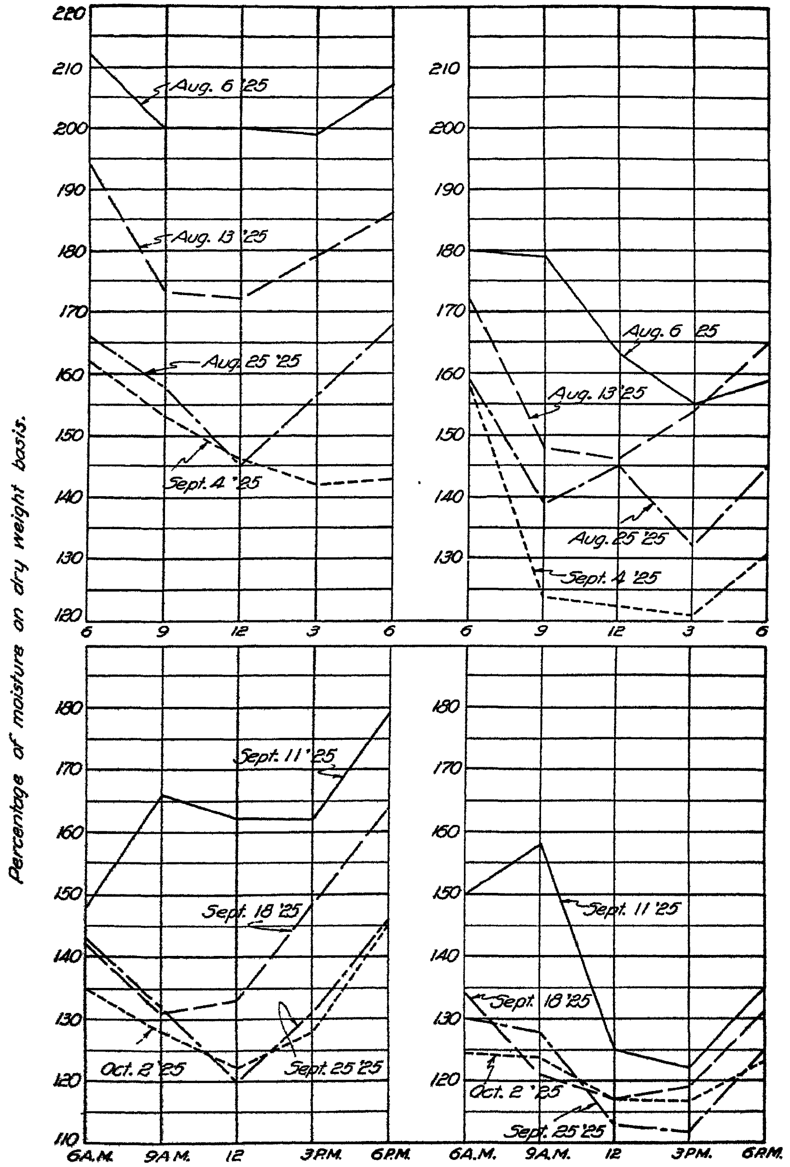

Fig. 11. Curves showing fluctuation in water content of bark from terminal six inches of current season's shoots on peach trees. Results from trees in moist soil are shown on the left; from trees in dry soil, on the right. The upper curves show results obtained at Davis; the lower, at Delhi.

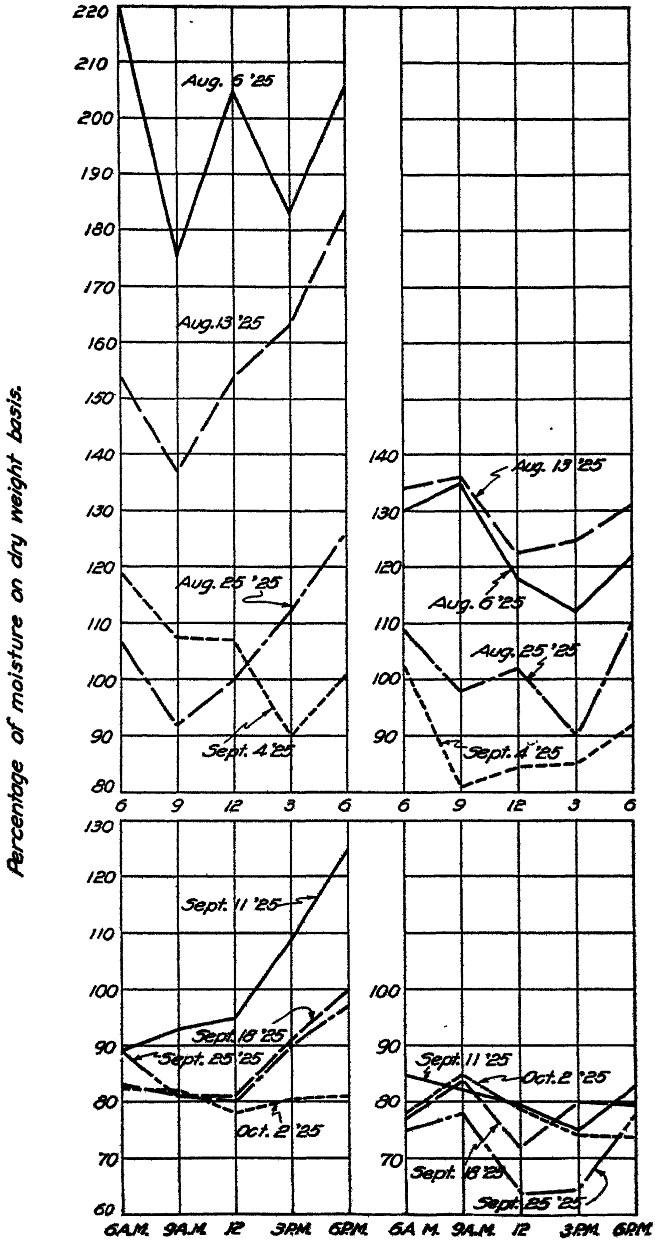

Fig. 12. Curves showing the fluctuation in water content of wood from the terminal six inches of current season's shoots on peach trees. Results from trees in moist soil are shown on the left; from trees in dry soil, on the right. The upper curves show results obtained at Davis; the lower, at Delhi.

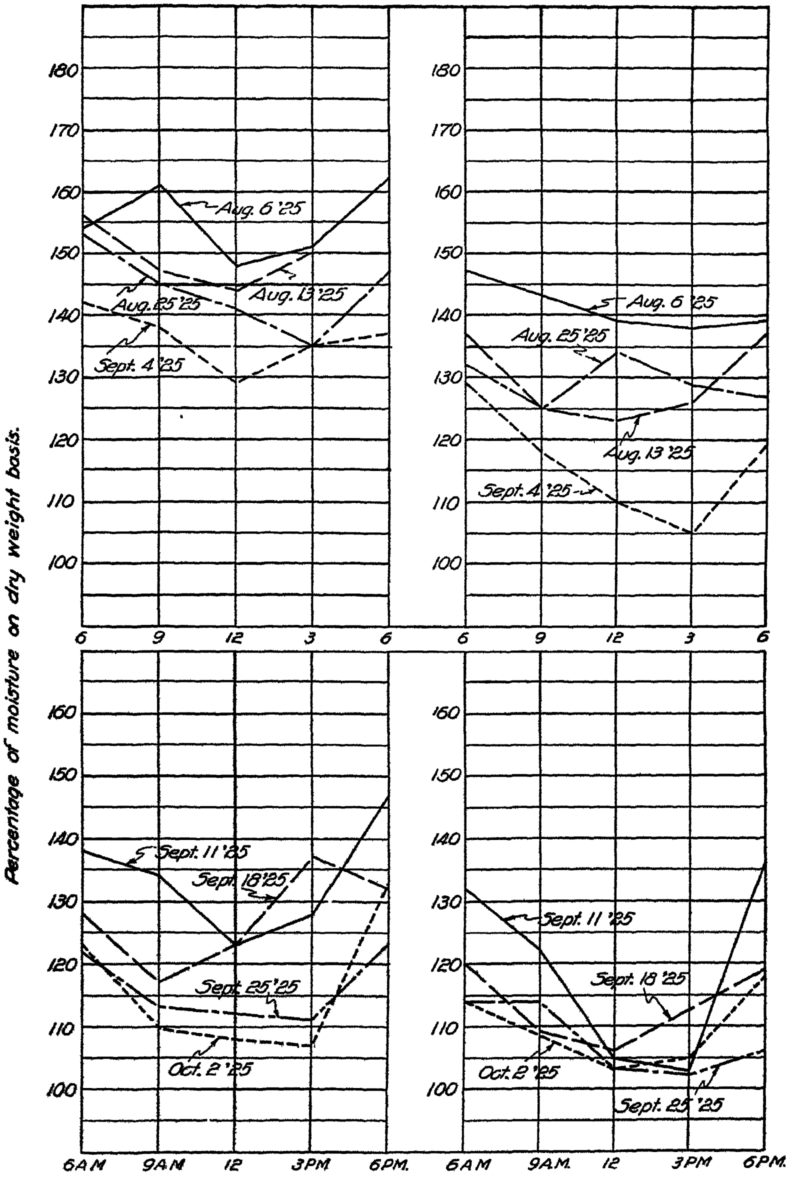

Fig. 13. Curves showing the fluctuation in water content of bark from the basal six inches of current season's shoots of peach trees. Results from trees in moist soil are shown on the left; from trees in dry soil, on the right. The upper curves show results obtained at Davis; the lower, at Delhi.

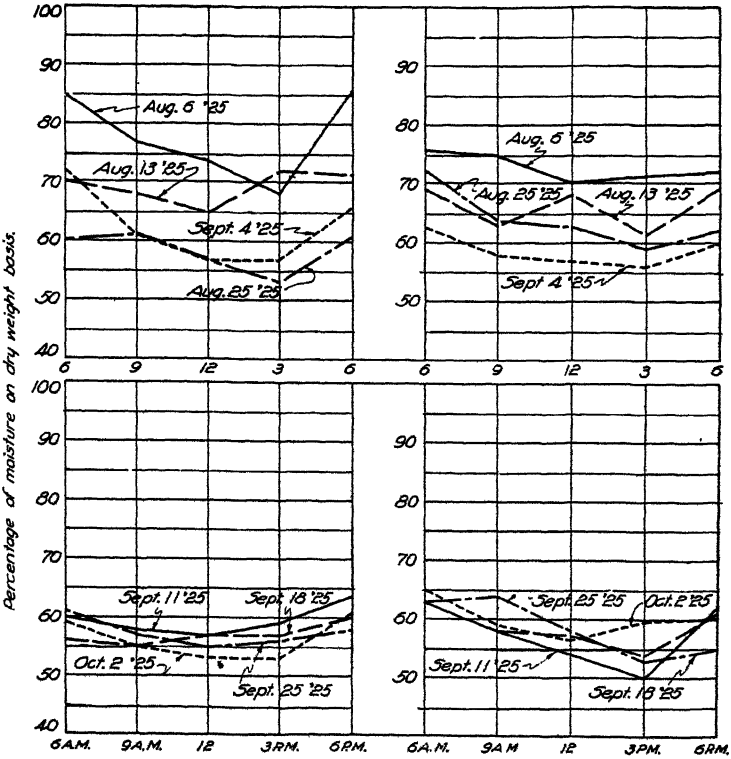

Fig. 14. Curves showing the fluctuation in the water content of wood from the basal six inches of current season's shoots on peach trees. Results from trees in moist soil are shown on the left; from trees in dry soil, on the right. The upper curves show results at Davis; the lower, at Delhi.

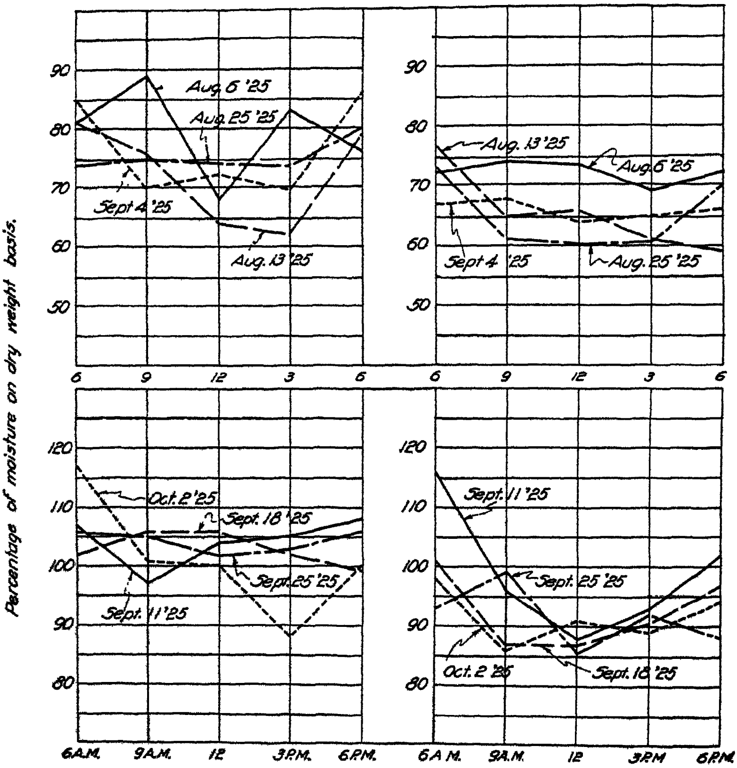

Fig. 15. Curves showing the fluctuation in water content of bark from the trunk of peach trees. Results from trees in moist soil are shown on the left; from dry soil, on the right. The upper curves show results obtained at Davis; the lower, at Delhi.

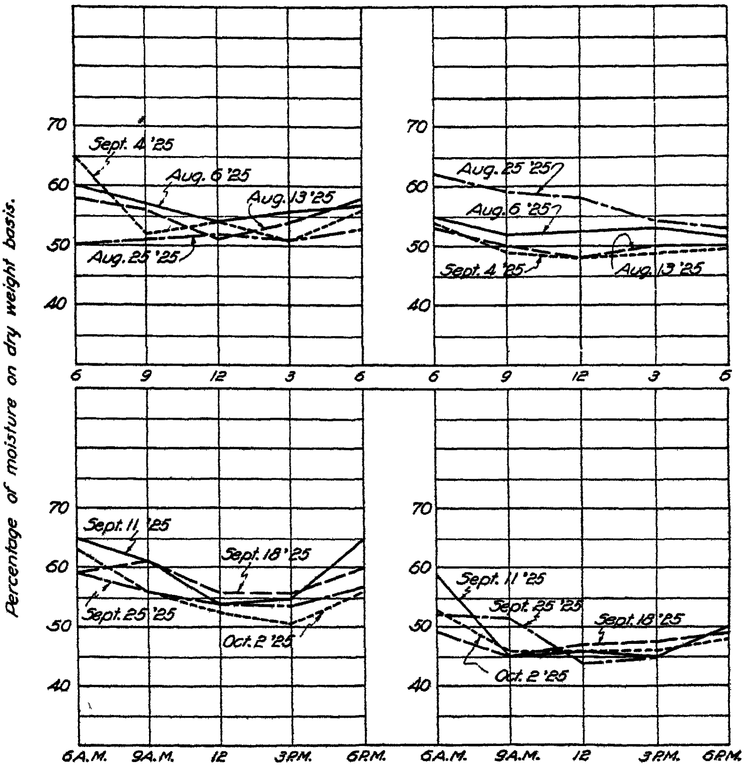

Fig. 16. Curves showing the fluctuation in water content of wood from the trunk of peach trees. Results from trees in moist soil are shown on the left; from trees in dry soil, on the right. The upper curves show results obtained at Davis; the lower, at Delhi.

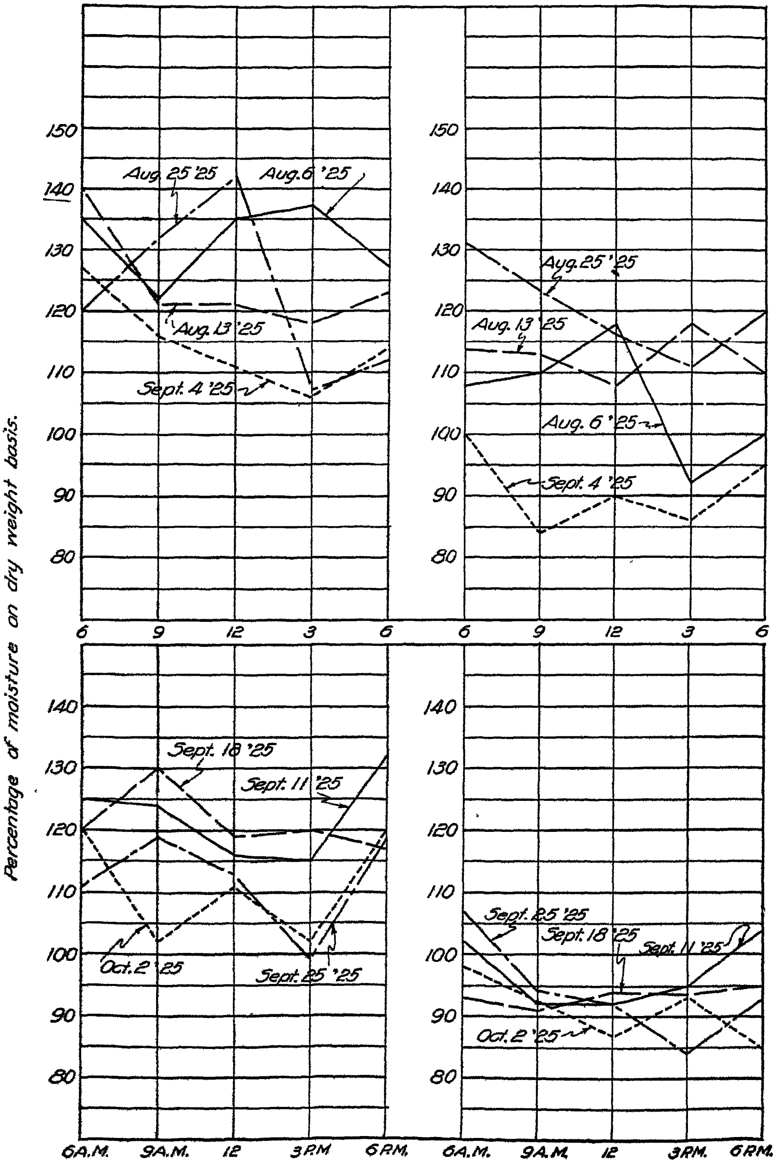

Fig. 17. Curves showing the fluctuation in water content of bark from the roots of peach trees. Results from trees in moist soil are shown on the left; from trees in dry soil, on the right. The upper curves show results obtained at Davis; the lower, at Delhi.

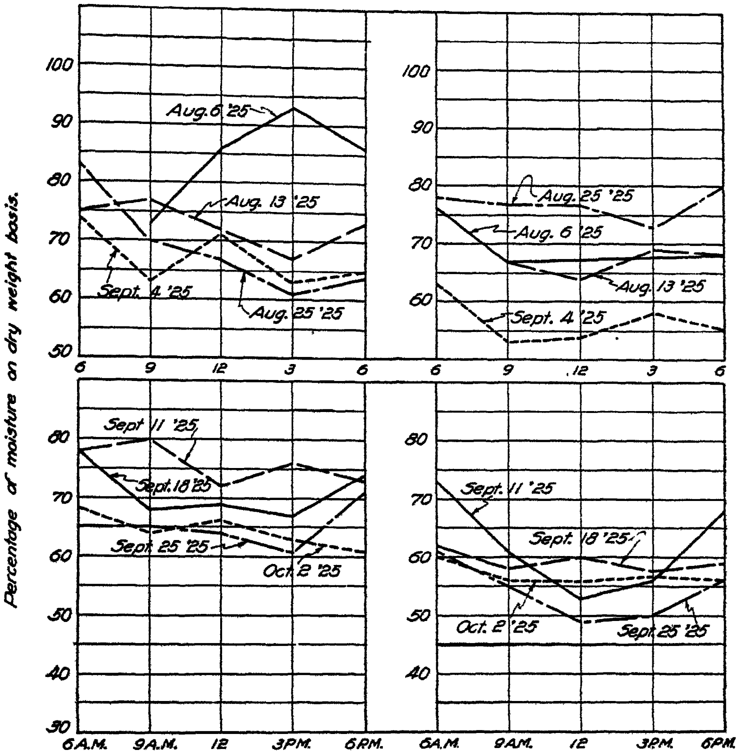

Fig. 18. Curves showing the fluctuation in water content of wood from the roots of peach trees. Results from trees in moist soil are shown on the left; from dry soil, on the right. The upper curves show results obtained at Davis; the lower, at Delhi.

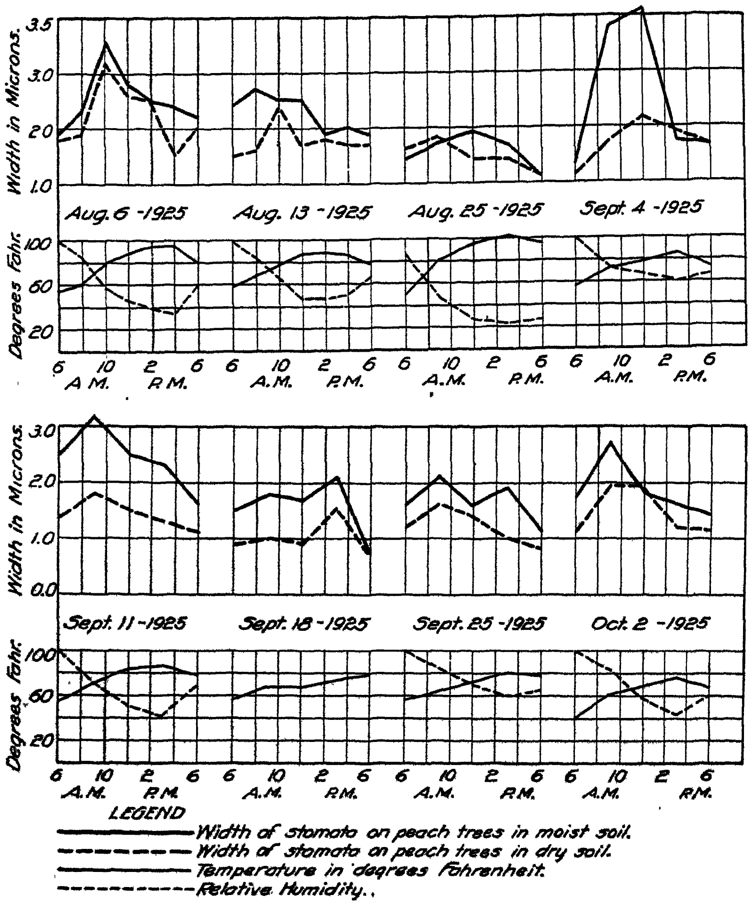

Fig. 19. Curves showing the behavior of stomata on peach leaves at Davis and at Delhi, during August and September, 1925. The leaf samples from which the stomatal measurements were made, were taken on the same day the samples of wood and bark were secured.

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